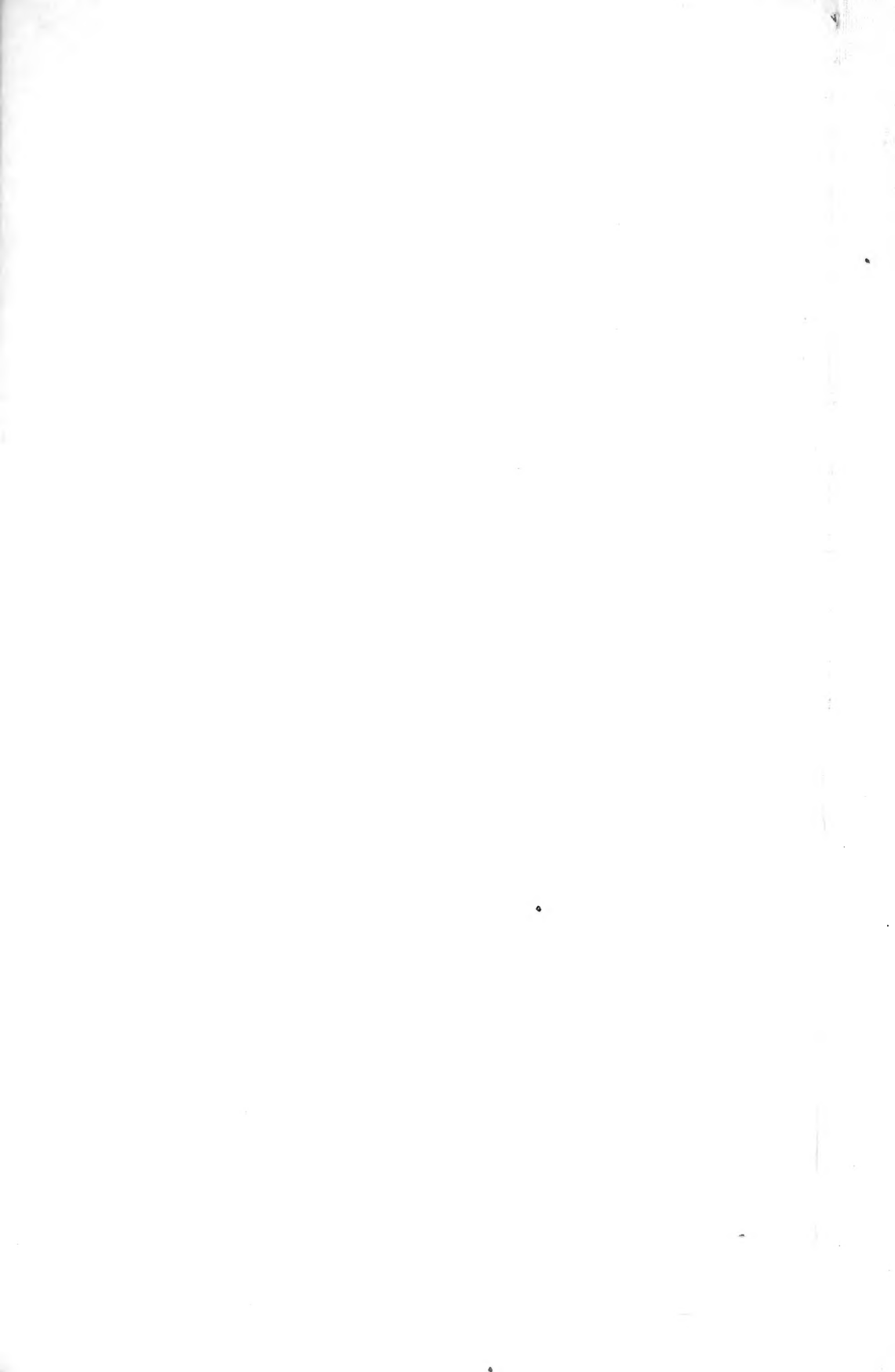


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THE JOURNAL

OF

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VOLUME 18

1915

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

PHILADELPHIA, PA.

137856
22/2/16.

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v. 18
cop. 2

COMPOSED AND PRINTED AT THE
WAVERLY PRESS
By THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

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THE GROWTH OF ORGANS IN THE ALBINO RAT AS AFFECTED BY GONADECTOMY

SHINKISHI HATAI

The Wistar Institute of Anatomy and Biology

The object of the present paper is twofold. (1) To test the several findings given in my previous work on the effect of gonadectomy (Hatai '13) and (2) to extend similar observations to several other ductless glands besides the hypophysis and ovaries. Furthermore, the present paper includes observations on the effect of semi-castration, ligation of the spermatic cords, and isolation of the ovaries from the uterus. In some series the weight and length of various bones, as well as the weights of parts of the encephalon, have been investigated also.

For this discussion I have found it convenient to introduce the word 'gonadectomy;' a word proposed to cover both castration and spaying. As the literature shows, the word 'castration' is often used for operations on both males and females, while in some instances castration is applied only to operations on the males; and for those on the female the word 'ovariotomy' or 'spaying' is used. This double use of the word 'castration,' signifying either the removal of the testes alone, or the sex glands of both sexes, makes for confusion.

The term 'gonadectomy' is useful therefore not only because it reduces to one word the expression "removal of the sex glands in either sex" but also because it leaves 'castration' to apply to the operation on the male, while that on the female is designated by 'spaying.' In this paper, therefore, the word gonadectomy is used throughout, whenever reference is made to the removal of the sex glands in both sexes, or without distinction of sex.

MATERIAL

The albino rats which have been used were obtained from the colony at The Wistar Institute during 1912 to 1913. The individuals belonging to the same litter were divided into two lots; one lot for control and the other for operation. The comparison is thus made always within the litter. This method of comparison is the best, since as shown by Jackson ('13) the variability of the body weight in the litter is only about one half that in the total population. It is further the impression of the present writer that peculiarities, when present, are usually found in most of the members of the same litter.

In the majority of instances the rats were subjected to operation at the age of 20 to 30 days, but in some series the operation was done at three different age periods with a view to determining the effect of age on the results. All the operations were performed by Dr. Stotsenburg at The Wistar Institute and I wish to acknowledge with thanks his courtesy in this matter. The method of the operation has been published by Dr. Stotsenburg ('09). The method for removal of the various organs after death is given in my papers published recently (Hatai '13, '14) and any other methods which have not already been given there will be described later.

Usually the operated rats were placed in the same cage with the controls. Thus one cage ($1 \times 1 \times 5$ feet) was occupied by all the individuals belonging to a given litter. If, however, the litter comprised more than six individuals, it was divided between two cages. The operated and control animals were thus treated similarly during the period of observation, and furthermore, the normal rats in the litter were allowed to breed. The object of doing this was to keep the rats under as natural conditions as possible.

Since we already have sufficient data on the growth of the body in weight following gonadectomy, the rats used for the present investigation were not weighed while under observation. The operated and control rats were examined on the same day unless the appearance of disease necessitated an early killing of one or the other.

FORMULAS

In order to determine the amount of deviation of any character (e.g., the weight of the organ, external measurements, etc.) in the operated animals from the same character in the controls, there are two methods which are commonly used:

1. The weights of the organs (for example) belonging to both the controls and operated are directly compared, irrespective of the difference in the body weights or ages of the animals employed. So long as the weight of the organ varies with the weight of the body or with the age of the animal, this method is obviously misleading, nevertheless, it has been used by a surprisingly large number of investigators.

2. The second method, which is more commonly employed, is to determine the ratio between the weight of the organ and that of the body and then to compare the magnitude of the ratios for both the controls and the operated. This method is, however, not always satisfactory and indeed in some cases may even lead to an entirely wrong conclusion when the relation between the body weight and the organ weight during growth deviates widely or irregularly from linearity. It is evident, therefore, that the safer method is to make a preliminary study of the phases of the normal growth of the organ or organs of the species in unoperated individuals in order to obtain data by which to estimate the relative deviations more correctly, or at least to avoid any misinterpretations arising from the peculiarities in the growth of the organs with respect to either body weight, body length or age.

Fortunately, in this laboratory we have at hand for the albino rat very adequate data on all the organs and characters under consideration, and furthermore from these data the mathematical formulas have been worked out in order to facilitate interpolation, as well as to study the forms of the growth curves for these characters.

I shall now indicate the method I have employed of computing for any animal examined, the magnitude of the deviations by the use of the formulas. All the formulas which are used in the

present investigation have been given in my papers recently published (Hatai, '13, '14) and therefore they will not be repeated here.

In determining the deviation of the organs, I have chosen the body length as the basis for the computation since this is the character of the body least subject to incidental modification. As a first step, the weights of the various organs corresponding to the observed body length of the rats under examination are computed by means of formulas. This computation is made for both the controls and the operated animals. The computed values thus obtained usually differ from the observed values, though this difference is not always significant. The differences between the observed and computed values of both operated and controls are now transformed into percentages by taking the computed values as 100 per cent. Thus we obtain two sets of percentage values, one expressing the difference between the computed and observed values in the control rats, and the other expressing the differences between computed and observed values in the operated rats. If the operation has not altered the organs of the animals at all, then these two sets of percentages should be alike within the limits of the normal fluctuations. If, on the other hand, the operation has altered the organs, these two sets of percentages should differ more or less according to the nature of the response to gonadectomy. If we now take the differences of these percentage values as given by the controls and by the operated rats, these differences represent the values by which the operated animals depart from the controls: the body length being used always as the standard for the computations. I have given in table 1 an illustration of the process of computation as here explained.

It must be stated, however, that in the case of the thymus gland, age is taken as the basis for the computation, since the weight of the thymus is much more highly correlated with the age of the rat than with either the weight or the length of the body (see Hatai '14). Again, in the case of the percentage of water in the central nervous system, I have taken the differences directly from the observed percentage values, since the per-

centage of water is a function of the age of the animal (subject to a slight correction for the absolute weight of the central nervous system—Donaldson) and hence so long as the ages of the control and operated rats are identical, this direct method is sufficiently accurate.

CASTRATION OF ALBINO RAT

CASTRATION AT 16 TO 22 DAYS OF AGE

This experiment is based on 16 litters comprising 27 controls and 26 operated rats. These 16 litters have been distributed in 6 groups, each containing two or more litters. In all cases the grouping was so made as to bring together those in which the average body lengths of the controls were most similar. The animals were examined 138 days after operation and the results of the observations are given in table 2.

Body length. If we compare the observed body lengths of the control and the castrated rats, it is noted that the castrated have a body length slightly less than that of the controls. This occurs in all six groups and gives an average difference of 4 per cent in favor of the controls. In the two previous studies (Donaldson and Hatai '11, and Hatai '13) we also noticed a similar reaction, that is the body lengths were absolutely shorter in the castrates than in the corresponding controls. We conclude therefore that the castration of young rats hinders the growth of the body in length.

Tail length. It has been noted in my previous study (Hatai '13) that, with respect to the body length, the tail lengths of the castrated rats are slightly greater than those of the controls. The average difference was 5.2 per cent in favor of the castrates. We notice in the present experiment the same degree of deviation; that is, the tails of the castrates are on the average 4.7 per cent longer than those of the controls.

We did not determine the tail length for the rats of the 1909 series. As the tail of the female rat is normally slightly longer than that of the male, this modification of the tail length brings the castrated male rats nearer to the female in this respect.

Since the anal opening is taken as the point dividing the body from the tail, the question arises whether the longer tails in the operated rats might have been due to the shifting of the anus following removal of the testes, the length of the caudal vertebrae not being altered. As will be seen later, the tail length of the rats whose spermatic cords had been ligated are also longer than those of the controls, to the same extent as in the castrates, despite the fact that these rats have the scrotum of normal size (though filled mostly with fat and connective tissue) and in the normal position. This seems to indicate that in the castrates the elongation of the tail with respect to the body length should not be attributed to a mere shift of the anus. Whatever be the real cause of this phenomenon, we note with interest the definite alteration of this external measurement as a result of castration.

Body weight. The absolute body weights of the castrated rats are slightly below those of the controls. This is to be expected since the body length, which is correlated with the body weight, is less in the castrates than in the controls. When, however, the body weights in the two series are compared with respect to the body length, the castrates show a relatively heavier body weight, though the average difference is only 6 per cent above that of the controls. This difference is certainly small when the normal amount of the fluctuation is considered, nevertheless it occurs in five out of the six groups, and therefore it should not be neglected. In my previous study (Hatai '13) the corresponding difference in favor of the castrates was found to be 3.6 per cent. It seems therefore probable that there is some tendency to a slight overgrowth in body weight with respect to the body length as the result of castration. Although precise tests have not been made, this increase does not seem to be due to an excess of fat, as in the case of the spayed rats, since the post mortem examination shows no evident difference in the fat deposition in the two groups.

Ductless glands

Thyroid gland. Table 2 shows that the thyroid of the castrates weighs less than that of the controls. The average deficiency is 21.5 per cent. This deficiency occurs in five groups out of the six. It should be noted, however, that the difference ranges from -0.8 per cent to -90.3 per cent and this wide range of variation must undoubtedly be taken into consideration as the normal variability of the thyroid gland is very high. For this reason an interpretation of the results will be postponed until the relations of the thyroid in the remaining series have been presented.

Suprarenal glands. We notice that these glands show a regular increase in the castrates as compared with the controls. On the average this amount is 17.6 per cent. The amount of the deviation is quite regular, being from 12.8 per cent to 21.4 per cent. Furthermore, the increase is shown in all six groups, thus indicating the significance of the results despite the rather moderate magnitude of the alteration.

Thymus gland. The thymus gland and hypophysis are the two organs which exhibit the most striking reactions after castration. As is shown in table 2, the thymus of the castrates is on the average 61.2 per cent greater than that of the controls. The range of variation is quite uniform with the exception of one undoubtedly abnormal instance (group X) and extends from 63.5 per cent to 87.4 per cent. If we neglect the unusual instance of 3.2 per cent in Group X, we obtain an average increase of 72.7 per cent instead of 61.2 per cent, as given in table 2.

Hypophysis. The hypophysis also shows a striking alteration as the result of castration. This amounts on the average to 56.7 per cent. The increase is present in all the groups and its value ranges from 45.0 per cent to 67.0 per cent. The present experiment confirms fully the results obtained from the castrated rats during 1912 (Hatai '13) where the increase in the hypophysis was found to be 75.6 per cent.

The somewhat smaller response in the present series may probably be correlated with a slight overgrowth of the body in

weight as contrasted with that found in the previous study. As will be seen later, there is a close reciprocal correlation between increase in the weight of the body including the fat and the increase in the weight of the hypophysis.

Central nervous system

Brain weight. As will be seen from table 2, the brains of the castrates show a slight but constant overgrowth when compared with these of the controls. The excess is 2.6 per cent on the average. We have noted in two previous studies a slight decrease instead of an increase, though the amount of decrease was by no means large. Thus we found in the castrates, -3 per cent in the 1911 series (Donaldson and Hatai '11) and -0.3 per cent in the 1913 series (Hatai '13). This lack of correspondence in the results suggests that the differences noted here may be fluctuations merely.

Spinal cord weight. Like the brain, the weight of the spinal cord shows fluctuations which are however slightly greater than those in the brain. On the average we find in this study an increase of 3.3 per cent in the cord weight in favor of the castrates. Further, this increase is shown in five out of six groups. In the 1910 series (Donaldson and Hatai '11) there was found a *deficiency* of 5.3 per cent; on the other hand, in the 1912 series (Hatai '13) an increase of 3.0 per cent, and finally in the present series an increase of 3.3 per cent. In addition to these results just mentioned, the values obtained from other experiments which will be described, suggest that the results are probably mere cases of fluctuation, and not to be causally related with gonadectomy. This subject will be discussed later.

Water content in the brain and in the spinal cord. The water content of the central nervous system is practically unaltered by castration. We note from table 2 the difference of 0.01 per cent in the case of the brain and 0.20 per cent in the case of the spinal cord, both in favor of the castrates. This result agrees with those of the two previous series, and thus it seems safe to

conclude that the percentage value of the water content of the central nervous system is not altered by the removal of the testes.

In order to determine the effect of age on the results of castration, a small number of rats were operated upon at two other periods: one lot at 79 to 93 days and the second lot at 208 to 232 days. The results are shown in table 3 and table 4.

CASTRATION AT 79 TO 93 DAYS

This experiment comprised four litters represented by 6 control and 5 operated rats. These were examined 111 days after operation. We note from table 3 that the results obtained from the present series are not essentially different from those obtained where the rats were castrated between 16 and 22 days of age. In this series we shall comment only on the values obtained for the thyroid and for the central nervous system.

In the case of the thyroid, the increase is 3.8 per cent against -21.5 per cent after early castration, and in the case of the brain and spinal cord, the increases are 0.5 per cent and 0.3 per cent respectively as against 2.6 per cent and 3.3 per cent always in favor of the castrates. These results suggest strongly that the variations recorded in the previous series are probably mere fluctuations.

CASTRATION AT 208 TO 232 DAYS

This study comprises three litters represented by 7 controls and 8 castrates. The animals were examined at 273 days of age or 55 days after operation. The reactions shown by this oldest series are again similar to those of the two previous series. The *highest* fluctuations are shown by the thyroid and the central nervous system. The remaining characters show somewhat less difference between the controls and castrated than is found in the two previous series. This slight response may be due either to the shorter interval between operation and examination, or to the advanced age at operation which finds the animals less responsive.

We note with particular interest the average increase of the thymus gland (53.9 per cent) even at this advanced age. In the normal rat the thymus gland reaches its maximum weight at about 85 days and then begins to diminish in absolute weight very steadily. Indeed at 200 days its loss in weight from the maximum value is nearly 44 per cent (Hatai '14) yet even after this atrophy it appears that the thymus is capable of responding to castration to nearly the same degree as when the operation is performed on younger rats.

Parts of the encephalon

We have noted that the weight of the brain is not noticeably modified by castration, nevertheless it was thought possible that the relative weights of the different parts of the brain might have been modified. To test this the brain was divided into four parts: cerebrum, stem, cerebellum and olfactory bulbs, in the following way:

1. Olfactory bulbs. The brain is placed with the dorsal side up and with a very sharp scalpel the olfactory bulbs are cut from the rest of the encephalon by section of the olfactory tracts just caudad to the bulbs. The portions thus separated are designated as the olfactory bulbs.

2. Cerebellum. The cerebellum is next removed by severing with sharp scissors the several peduncles.

3. Cerebrum. The cerebrum is separated from the stem by cutting with a scalpel carried in the plane passing just in front of the dorsal edge of the anterior colliculi and just caudad to the corpus mammillare.

4. The remaining portion, caudad to this plane of section, is the stem.

The results of this investigation are given in table 5. The rats used for it belonged to the series castrated at 16 to 22 days. They were arranged according to age instead of body length, since the relative weights of the parts of the encephalon are more closely correlated with age.

The total number of the animals used was 22 control and 22 castrated, and these were examined in 6 age groups. In each group the controls and castrated belong to the same litters. As will be seen from table 5, there is practically no difference in the proportional weights of the parts of the brain in the two series. There is however some slight tendency to a relatively smaller weight of the olfactory bulbs in the castrates. The observations are too few and the difference too slight, however, to justify us in putting emphasis on the deviation now. I therefore conclude that so far as the observations go, there is no important difference between controls and castrates in the relative weights of the parts of the brain.

Weights and lengths of certain bones

In order to determine whether or not castration produces modifications in the skeletal system of rats, some bones, the humerus, radius and ulna, femur and tibia and fibula, were examined. The preparation of the bones was as follows:

The fresh bones were roughly cleaned by dissection and then put into a hot solution of 2 per cent "gold dust cleaning powder" (a commercial preparation) for nearly two hours. The period of course varies slightly according to the size and age of the specimens. As soon as the remaining soft parts were macerated, the bones were cleaned with a strong tooth brush, with an occasional use of the bone scraper. These bones thus cleaned were gently wiped with blotting paper and weighed. This weight is designated here as the 'fresh weight.' The bones were then placed in small vials without corks. After about eight months these bones were again weighed and the amount of moisture was determined—this gave the 'dried weight.'¹ It may be stated that we have had a large experience with the method of preparation in connection with other investigations in this

¹ This is not to be confused with the weight of 'dry substance,' to be obtained by subjecting the bones to a temperature of 95° C., until they reach a constant weight.

laboratory, and the above method has been found to be perfectly trustworthy for purposes like the present.

The method just given has been applied to all the other series (after spaying and semi-spaying) in which the bones have been studied. The results of this examination are given in table 6. The final averages from table 6 show the following relations:

	BODY LENGTH TO AV. BONE LENGTH	BODY WEIGHT TO BONE WEIGHT	WATER CONTENT IN PER CENT
Controls.....	1:0.141	1:0.0124	25.14
Castrated.....	1:0.142	1:0.0129	27.29

We note from these relations that the difference between the controls and the castrates is very slight, except in the water content in which a difference is clearly indicated. It must be noted, however, that when examined by litters there is a tendency, though slight, for the castrated rats to give constantly a small relative excess in both length and weight of the bones. The small excess is shown in all four groups. At the same time it must be admitted that the difference is certainly much smaller than might have been anticipated from the reports of previous investigators who have discussed the modification of the skeleton after castration.

SPAYING OF THE ALBINO RAT

SPAYING AT 19 TO 30 DAYS OF AGE

The present observations are based on 15 litters comprising 30 controls and 28 spayed. These 15 litters were arranged in 6 groups according to the method adopted for the previous series. The animals were examined from 101 to 190 days after the operation. The results are shown in table 7.

Body length. If we compare the absolute values of the body lengths of the control and spayed, we note a slightly longer body in the spayed. Though the difference is only 2.5 per cent in favor of the spayed, nevertheless it occurs in five out of six groups,

while the remaining group shows equality. The significance of this difference is enhanced, despite its small value, by the fact that it has been observed in a previous study (Hatai '13) that the spayed show an increase of 2.7 per cent in body length over that of the controls. From these two studies, which present the same degree of increase, we may safely conclude that spaying stimulates the growth of the body in length. It is interesting to note in this connection that castration as already pointed out (see page 5) has the reverse effect.

Tail length. With respect to the body length, the tails of the spayed are slightly longer than those of the controls. The average increase is 1.9 per cent in favor of the spayed. This increase, however, may not be at all significant, unlike the reaction in the case of the castrates, as it is highly irregular. Moreover, the two other spayed series, which will be presented later, show a decrease in one case and an increase in the other in the length of the tail. These results can best be taken as indicating a statistical variation rather than a significant modification produced by the removal of the ovaries.

Body weight. The body weight with respect to the body length is greater in the spayed than in the controls. The average value gives an increase of 7.3 per cent in favor of the spayed. The range of this increase is high, being from 2.2 per cent to 13.2 per cent, nevertheless it is always in the same direction; that is the spayed have a relatively heavier body. The same response was noted in my previous study on spaying (Hatai '13) though the difference there found was only 3.1 per cent. This low value obtained from the 1912 series was due to the somewhat emaciated condition of some of the rats at the time of killing. We conclude, therefore, that the spayed rats are longer and heavier, while the tail length is not altered by spaying. It should be added here that the spayed rats belonging to the present series did not show as much obesity as those belonging to some previous series (see Stotsenburg '13, and Hatai '13). This fact is important since the variations in the degree of obesity are intimately related with the variations in the weight of the hypophysis.

Ductless glands

Thyroid gland. As in the case of the castrates, the thyroid gland of the spayed shows considerable fluctuation in weight. Although on the average spayed have a slightly heavier thyroid (3.3 per cent) nevertheless we find an increase in three groups and a decrease in the remaining three, and in addition to this inconstancy the range of variation is also very great. We shall therefore postpone the discussion of this to a later occasion.

Suprarenal glands. The suprarenal glands of the spayed are smaller than those of the controls. The difference on the average is 18.5 per cent against the spayed. This diminution in the spayed occurs in all six groups, while the range of variation is not so great if we omit one instance, giving an exceptionally small value (group 9, in which the difference is -5.7 per cent). Furthermore, the two remaining spayed series also show a corresponding diminution and thus we may safely conclude that spaying hinders the normal rate of growth of the suprarenal glands in respect to the body weight.

It is interesting to recall that castration tends to increase the weight of the suprarenal glands—just the opposite effect to that produced by spaying. Since normally the female rat has larger suprarenals than the male, and since castration increases, while spaying diminishes the weight of these glands, we see that as the result of gonadectomy the two sexes tend to approach one another in this character.

Thymus gland. The thymus gland of the spayed shows a most striking alteration. In some cases its increase runs as high as in the castrates, and indeed on the average it shows an increase of 58.3 per cent in favor of the spayed. This increase in the spayed occurs in all six groups, and thus the causal connection between the greater size of the thymus and removal of ovaries is clearly shown. The range of increase, 34.2 per cent to 75.8 per cent, was higher than might be anticipated on account of the two groups in which the increase was rather small.

Hypophysis. The hypophysis shows a distinct increase of 8.9 per cent in the spayed rats. This increase ranges in the

several groups from 0.3 per cent to 21.6 per cent. However, it occurs in all the groups and furthermore it has been noted in my previous study (Hatai '13). It is interesting to observe that in general the increase of the hypophysis is negatively correlated with gain in body weight.

Central nervous system

Brain weight. The relative brain weight of the spayed rats is slightly heavier than that of the controls. On the average the difference is 1.3 per cent, and it occurs in five out of six groups. The other two studies on spayed rats, to be presented later, give in one study similar values, and a decrease in the other. These irregularities suggest that the increase recorded in the present series should be regarded as a fluctuation.

In my previous study (Hatai '13) the spayed rats showed a decrease of 0.4 per cent in the weight of the brain. This disagreement between the results of two successive years supports the view that we are here dealing with a fluctuation only.

Spinal cord weight. The spinal cord of the spayed rats shows a still greater relative increase than does the brain. On the average, the spayed shows 2.5 per cent increase, and furthermore this occurs in five out of the six groups. A slight increase in the spinal cord weight has also been observed in a previous study (Hatai '13) and in view of the fact that the increase occurs in all operated animals (see all the experiments reported in this paper) we are hardly justified in regarding it as a mere fluctuation, though at the same time there does not seem to be any reason to expect this increase. I shall therefore leave this point without attempting any interpretation at the present time.

Percentage content of water in the central nervous system. The amount of water present in both the brain and spinal cord is not altered by the removal of the ovaries. We note from table 7 only an insignificant difference between the control and spayed rats. This result agrees fully with that of the two previous studies and we may conclude therefore that the water content of the nervous system remains unmodified. In addition I have

studied two other series represented by the albino rats spayed at more advanced ages: (1) Spayed at 97 to 119 days and (2) spayed at 172 to 195 days of age.

AFTER SPAYING AT 97 TO 119 DAYS OF AGE

This series comprises five litters containing 9 controls and 8 spayed. These rats were examined 100 days after spaying. The results of the operation are shown in table 8. We find in this series a general agreement with the previous results.

Body length. The absolute value of the body length of the spayed is slightly greater than that of the controls. This agrees with the previous observation.

Tail length. The relative tail length of the spayed is slightly shorter (1.5 per cent) as against the slight increase in the previous series. This disagreement suggests that the variations in tail length are merely fluctuations.

Body weight. The body weight of the spayed rats shows a slight relative increase and thus agrees with the previous observations.

Thyroid gland. The thyroid of the spayed shows 50.6 per cent decrease as against 3.3 per cent increase in the previous series. This disagreement indicates that the variations shown by this organ are probably fluctuations or dependent on conditions not yet recognized.

Thymus gland. We note 39.6 per cent increase in the spayed. This result agrees with the previous observations.

Hypophysis. The hypophysis of the spayed shows 7.8 per cent increase. The results agree with the previous observations.

Brain weight. As before, there is no difference in the weight of the brain between the controls and the spayed.

Spinal cord weight. There is a very slight increase of 1.5 per cent in the weight of the spinal cord of the spayed. This agrees with the previous observations.

The percentage of water in the central nervous system shows no significant variations.

AFTER SPAYING AT 172 TO 195 DAYS OF AGE

This series comprises three litters containing 9 controls and 7 spayed rats. The rats were examined 27 to 60 days after operation. The results are shown in table 9. This series shows alterations essentially similar to those found in the two preceding series, and therefore a detailed description may be omitted.

We note with special interest the reaction shown by the thymus gland after spaying. It has been stated already that the thymus gland at 200 days is nearly 44 per cent less than at the maximum stage (at 85 days). Thus even in this advanced stage of involution the thymus gland responds to spaying and shows an average increase of 27.5 per cent. In one case, group 14, the increase is as high as 60.8 per cent, or almost as great as in the rats operated at 19 to 30 days. All the other characters show the alterations we should expect from the two preceding series.

Parts of the encephalon

The parts of the encephalon of the spayed rats have been examined to see whether or not any differences in the relative weights between the spayed and controls can be detected. The methods of dissecting the brain, as well as of grouping the rats, were the same as used in the case of castration. The results of the investigation are given in table 10. As will be seen from the table, there is no significant difference in the relative weight of the parts of the brain in the spayed and in the controls. If we examine the different groups we note some tendency to relatively small olfactory bulbs in the spayed. The difference, however, is too slight to be regarded as of value. We conclude therefore that removal of the ovaries has no marked effect on the relative weights of the parts of the encephalon.

Weights and lengths of certain bones

The weights and length of the bones of the spayed, together with those from the controls, were investigated. The technique used and bones examined were the same as in the case of the castrated rats. The results of the investigation are given in

table 11. From the final average given in the table we find the following relations:

	BODY LENGTH TO AV. BONE LENGTH	BODY WEIGHT TO BONE WEIGHT	WATER CONTENT IN PER CENT
Controls.....	1:0.146	1:0.0134	23.44
Spayed.....	1:0.148	1:0.0128	25.35

From the above it appears that the differences in the relative values between the controls and the spayed are very slight. Nevertheless, we note as in the case of the castrates, that the spayed give a slightly higher value in the case of the bone length. In the case of the bone weight, the spayed rats give a less value. In this instance the weight relation is due to the abnormal fat deposition which follows spaying. According to the formula, a body length of 188 mm. (that of the spayed) should give a body weight of 170 grams, while the observed body weight of the spayed is 186.1 grams, thus showing a difference of more than 9 per cent. If therefore we take a corrected body weight of 170 instead of 186.1 grams, the ratio between the body and bone weight becomes 1:0.140, or the spayed have a slightly heavier relative bone weight than that of the controls.

The water content is distinctly greater in the spayed, thus agreeing with what was found for the castrates. We conclude then that gonadectomy in the rat tends to give rise to very slightly longer and heavier bones, which in turn have a higher water content than those of the controls.

SEMI-CASTRATION OF THE ALBINO RAT

AFTER SEMI-CASTRATION AT 17 TO 27 DAYS OF AGE

This series comprises 15 litters containing 22 controls and 24 semi-castrated rats. These 15 litters were placed in five groups according to the method previously employed. The results are given in table 12.

Body length. The semi-castrated rats did not grow in length as much as the controls. In this respect the semi-castrated are

similar to the castrates. On the average we find that the semi-castrated give a body length of 209 mm. against 217 mm. for the controls; a difference of 3.7 per cent in favor of the controls. This relation is true not only on the average, but appears also in four out of the five groups. In view of these uniform results, we draw the tentative conclusion that semi-castration hinders slightly the normal growth of the body in length.

Tail length. In four out of five groups the tail length of the semi-castrated is greater than that of the controls. On the average, the difference is 1.9 per cent in favor of the semi-castrated. It is evident that the amount of increase is hardly one third of that given by the castrates, and further the fluctuation is much greater than that of the castrates. Moreover, since we find a decrease in the relative length of the tail in the semi-castrates given in the next series, we conclude that the differences represent mere fluctuation, and that the length of the tail is not modified by semi-castration.

Body weight. The relative body weight is slightly greater in the semi-castrated than in the controls. We find on the average a difference of 1.9 per cent in favor of the semi-castrated. The increase is shown in five out of six groups, and further the range of variation is not large. This seems to be an instance of a slight tendency to an increase of the body weight as the result of the operation.

Testes. The surviving testis of the semi-castrate shows a distinct and uniform increase in weight over that of the normal control testes. The increase is on the average 14.2 per cent. This is also shown in all the groups and ranges from 6.1 per cent to 23.8 per cent. It is important to decide whether this increase is represented by the germinal tissue or by the interstitial tissue, or by both. It is generally held that the structure which is responsible for the internal secretion of the testis is the interstitial and not the germinal tissue. If this be true, and if we assume that the increase in this instance is represented by the interstitial tissue alone, then the percentage increase of the latter structure will be considerably larger than that indicated

by the values given here. We shall take up this subject later on, utilizing Hofmeister's observations ('72) on the interstitial tissue of the human testes.

Ductless glands

Thyroid gland. The thyroid gland of the semi-castrated shows an average increase of 16.4 per cent above the controls. Here again, as in the previous observations on the thyroid, the range of variation is very great, being from -37.3 per cent to 41.0 per cent, and furthermore in the rats semi-castrated at 81 days, and which will be described later, the thyroid shows 29.6 per cent decrease instead of an increase. It is not therefore possible at the moment to correlate the changes in the thyroid with this operation

Suprarenal glands. The relative weight of the suprarenal glands shows a slight alteration in the semi-castrated, giving an average increase of 2.7 per cent in their favor. This low value is due to an exceptionally small response given by group 5 (-0.9 per cent). If we neglect this exceptional case, the average rises to 3.5 per cent. The increase in the semi-castrated is shown in four out of five groups, and further the range of variation is not so large, particularly if we neglect the very small value given by group 5. Nevertheless, the increase is too slight to warrant us in putting much weight upon it. However, some tendency to an increase as the result of semi-castration can not be denied.

Thymus gland. Curiously enough, the thymus gland of the semi-castrated rats shows an average *decrease* of 16.1 per cent. The decrease occurs not only in the final average, but also in four out of the five groups. The range of variation is not large in comparison with the variations of the thymus glands belonging in other series. It ranges from -12.8 per cent to -30.0 per cent against the semi-castrated. On the other hand, we find in the rats semi-castrated at 81 days 9.8 per cent increase of the thymus—the exact opposite—and thus the interpretation is at present impossible. This is the only instance in all the experiments recorded in the present paper in which a decrease of the thymus gland as the result of the operation has been found.

Hypophysis. The hypophysis shows an average increase of 10.1 per cent as the result of semi-castration. The range of variation is great and lies between 0.5 per cent to 29.8 per cent. Semi-castration at 81 days shows 0.25 per cent decrease, equivalent to no change (see table 13). In view of this high variability of reaction all we can note is a tendency to overgrowth of the hypophysis as one result of this operation.

Central nervous system

Brain weight. We find that the weights of the brain in the controls and semi-castrated do not show any significant difference. The average value gives — 0.5 per cent against the operated rats. There is perhaps a very slight tendency to reduction in the brain weight of the semi-castrated, as it occurs in four out of the five groups. However, the difference is in many cases less than one per cent and we thus conclude that semi-castration produces no important alteration in the weight of the brain.

Spinal cord weight. The spinal cord of the semi-castrated rats with respect to the body length is slightly but constantly larger than that of the control rats. Its increase is shown in all five groups and the final average shows 2.2 per cent in favor of the semi-castrated. This agrees with the results of all the experiments so far presented.

Percentage of water in the central nervous system. In this character the difference between the operated and control rats is very small. We therefore conclude that the proportion of water which is present in the central nervous system is not altered by semi-castration.

AFTER SEMI-CASTRATION AT 81 DAYS OF AGE

The number of rats comprised in this series is rather small (6 controls and 5 semi-castrated) but the results show a close agreement with those rats from which one testis was removed at 17 to 27 days. The data are presented in table 13. It is only necessary to point out in this series also that the surviving testis shows a typical increase in size. The increase is 15.1

per cent contrasted with 14.2 per cent for the previous series. The alterations shown by the other characters are similar to those found in the series operated at an earlier age, and therefore we conclude that the effects of semi-castration at 81 days are nearly the same as those found when the operation is made at 17 to 27 days, with the exception of the hypophysis, in which the rats operated at the earlier period show a slight excess.

Parts of the encephalon

The parts of the encephalon of the semi-castrated rats have been investigated. The technique and method of grouping are the same as before (see page 10.) The rats employed for this study were those semi-castrated at 17 to 27 days with their controls. The results are given in table 14. As will be seen from the table, there is no significant difference in the relative weights of the parts of the encephalon in the control and the semi-castrated. We therefore conclude that the relative weights of the different parts of the encephalon are not modified by this operation.

THE LIGATION OF THE SPERMATIC CORD

Since the removal of the testes produces striking modifications in the external characters, as well as in some organs, and since the removal of one testis alone does not produce any noticeable alterations, presumably owing to the compensatory growth of the interstitial tissues of the surviving testis, it was thought interesting to determine the effect of ligation of the spermatic cord, an operation which interrupts not only the vas deferens but also the accompanying vessels and nerves. Although this operation leaves the testes in place, not only may their growth be stopped, but they may undergo various atrophies or degenerative changes as a consequence of it. The operation was made on a small number of rats; 6 operated and 6 controls. The results are shown in table 15. The operated animals were allowed to live a little more than three months. Examination of results reveals the fact that we must treat the

data in two groups: Group 1 comprises those operated rats whose testes, though typical in form, are yet small, pale in color, semi-transparent and filled with an abundance of liquid. Thus group 1 shows an abnormal condition of the testes, though their form was normal. Group 2 comprises those operated rats whose testes have been nearly completely absorbed.

Group 1. In this group the testes of the operated rats show a decrease of 31.6 per cent, nevertheless the typical form was still maintained. Although no histological examinations have as yet been made, it appeared to me that the germinal tissues were probably completely absorbed, or at least altered beyond recognition. As will be seen from table 15, the characters usually affected by castration were not noticeably modified. This negative result suggests that the interstitial cells of the testes were very likely intact or might even have proliferated.

Group 2. On the other hand, in those operated rats whose testes have been nearly completely absorbed (-93.2 per cent) alterations characteristic of the castrated rats are plainly to be observed. A slightly longer tail, larger suprarenal glands, thymus and hypophysis, definitely supports this conclusion. We conclude, therefore, that ligation of the spermatic cord is similar to castration in the reactions which it causes, if the testes are absorbed, but its reaction is negative if the testes persist, even though they may be materially reduced in size. Under these conditions it is inferred that the germinal tissue has been destroyed, while the interstitial tissue remains either in its normal quantity or possibly is hypertrophied.

SEMI-SPAYING OF THE ALBINO RAT

AFTER SEMI-SPAYING AT 38 TO 51 DAYS OF AGE

This experiment comprises a rather small number of rats, and the discussion of the effects of the operation will be limited to those characters only which showed undoubted alterations. The agreement in the results between the present series and that previously studied (Hatai '13) is clear. The results are shown in table 16.

In general, we may say that the semi-spayed show no significant response except in the increased weight of the surviving ovary. The large difference shown by the thyroid gland must for the present be credited to its high variability. The slight increase of the other ductless glands may be considered a general reaction common to all operated rats. On the other hand, the weight of the surviving ovary is most striking, showing an average increase of 131.8 per cent. Thus the one ovary of the semi-spayed weighs more than twice the normal ovary. In my previous experiment the increase of the surviving ovary was found to be 73.1 per cent; the present experiment thus gives the greater increase. No explanation for the amount of increase in the surviving ovary can be given at the moment, and we must be content to call attention merely to the striking character of the reaction.

The lengths and weights of the long bones, together with their water content, after semi-paying

The weights and lengths of these bones, together with their content of water have been investigated. The technique used is the same as that employed in the preceding cases. The results are given in table 17. From the final averages in the table we obtain the following relations:

	BODY LENGTH TO AV. BONE LENGTH	BODY WEIGHT TO BONE WEIGHT	WATER CONTENT IN PER CENT
Controls.....	1:0.140	1:0.0141	26.985
Semi-spayed.....	1:0.140	1:0.0128	26.977

From the above we find that the relation of the body length to the bone length is identical in the operated and controls. The ratio of the body weight to the bone weight is distinctly lower in the semi-spayed rats. This is undoubtedly due to the emaciation of the body weight of the control rats, as can be seen from the relation given by the length and weight of the body. Thus if we take the ratio between body length and bone weight, we

obtain 1:0.0126 in the case of the control and 1:0.0125 in the case of the semi-spayed. It is therefore safe to conclude that as compared with the controls, semi-spaying does not modify the bones either in weight or in length with respect to the weight and length of the body. We note also the practical identity in the water content of the bones in semi-spayed and controls.

THE ISOLATION OF THE OVARIES

Since the complete removal of the ovaries alters seriously several bodily characters and organs, and since semi-spaying does not produce any noticeable alterations, owing probably to the compensatory growth of the surviving ovary, it was thought interesting to determine the effect of the removal of the one ovary, combined with a severance of the other ovary from the uterus. This experiment has been made with a small number of rats and the results are shown in table 18.

As will be seen from this table, the result of the operation is not materially different from the case of the semi-spaying. No significant alterations are produced in any characters except the weight of the ovaries. We find here an average increase of 51.3 per cent in the surviving ovary. The amount of increase is not so large as in the case of the semi-spayed just described. The smaller alteration is to be expected, as of course the ovary is in the first instance severed from its normal connections,—blood supply, etc., and thus subjected to unfavorable conditions. In some instances the ovary was enclosed in a capsule containing fluid, and these cases showed unmistakable signs of absorption, the color was pale and the lobulation wanting.

DOUBLE ISOLATION OF THE OVARIES

From the results just described, we should anticipate that the isolation of both ovaries from the uterus would not be followed by any marked alterations. It was thought best, however, to test this assumption and therefore the following short experiment has been made. The results are given in table 19.

As had been anticipated, no notable alterations were shown as the result of the isolation of both ovaries. The responses exhibited by the various characters are very small, and particularly when we consider the small number of rats in the series, these modifications can all be neglected. We however note with some interest a slight increase of the ovaries (14.0 per cent) in the operated rats, but whether this increase is a consequence of the operation or a mere fluctuation, cannot be decided without more evidence.

GENERAL DISCUSSION OF THE RESULTS OF GONADECTOMY

So far I have merely presented my results without either interpretation or comment, save in the case of the external characters. I wish now to discuss these results and if possible to interpret the alterations following gonadectomy.

For convenience I give in table 20 the percentage deviations of the various characters in the operated rats from those in the controls. The external characters, such as absolute body length and body weight, and also body weight and tail length relative to body length have been briefly considered in the case of each series, and appear to be particularly related to changes in the hypophysis. It is especially with the members of the endocrine system, so far as we have examined them, that we are at present concerned.

Thyroid gland. On the average the amount of deviation in the weight of the thyroid gland of the operated from that of the controls ranges from 34 per cent to -51 per cent (see table 20). On account this high variability, as well as the absence of uniformity of response to the same operation, no definite conclusions can be drawn from our results touching the effect of gonadectomy on it. Turning to the literature, we find that Tandler and Grosz ('13) observed constantly a small thyroid gland in eunuchs. Biedl ('13) mentions finding a small thyroid in one castrated dog. Soli ('09) found a considerable variation in the weight of the thyroid in capons. Out of the nine pairs which he examined, four showed an increase and five a decrease in the castrated

compared with the controls. Soli however found a constant increase in the weight of the thyroid in the castrated rabbits (numbers are not given) when examined three months after castration, but this in turn was followed by a decrease, so that at the end of the experiment the castrated rabbits had smaller thyroids than the controls. Engelhorn ('12), on the other hand, found an hypertrophy of the thyroid after spaying in women and in rabbits. Thus, so far as the previous investigations are concerned, the effect of gonadectomy on the size of the thyroid is not determined.

In view of the great variability of the thyroid in weight in both men and animals, and in view of the small number of animals used by most investigators and the almost complete absence of data for the normal animal, as well as the inconstancy of our own results on the albino rat, we conclude that no general statement as to the effect of gonadectomy on the thyroid is justified at present.

The thyroid gland of the albino rat presents, however, several interesting features worthy of remark. I have determined (Hatai '13a) that the hypophysis and the suprarenals of the albino rat show distinct sexual differences in their weights. The thyroid gland, however, does not show any sex difference in weight. Moreover, for any given body weight, the weight of the thyroid is practically identical in the (domesticated) albinos and (the wild) norway rats, while the other ductless glands show distinct weight differences in these two forms. Thus in the albino the thyroid glands shows no response to the changes in the surroundings (domestication) nor variations according to the sex similar to some other ductless glands.

Whether the thyroid of the rat is physiologically less important than some of the other ductless glands cannot be decided from such simple observations, nevertheless it is of interest to note that the ill effects of thyroidectomy in the rat are remarkably slight (see Vincent '12). One might suspect that the high variability of the thyroid gland was due in part to pregnancy in the females. I have examined my data with this idea in mind, but have failed to find any correlation between pregnancy and

thyroid weight. As a matter of fact, the variability of the gland is as large in the males as in the females.

Suprarenal glands. The effects of gonadectomy on the size of the suprarenal glands have been studied by several investigators. Feodossiew ('06) found an enlargement of the suprarenals in spayed dogs. Von Schenk ('10) found histological evidence of hyperfunction of the gland in spayed rabbits. He further noted an enlargement of the gland as indicated by the fact that the areas of the sections in the operated are greater than those of the controls. Von Schenk, however, did not record any weights. Marrassini and Luciani ('11) noted in spayed guinea-pigs and rabbits an enlargement of the suprarenals.

As the result of castration, Soli ('09) found a diminution of the suprarenals in the chicks, and in guinea-pigs and rabbits. In the latter however the suprarenals soon after operation showed a very slight increase which was followed by a distinct diminution in a latter stage. Marassini and Luciani ('11) on the other hand found a considerable increase in castrated rabbits and in guinea-pigs when compared with their respective controls. Stilling ('98) found an enlargement of adrenals in male rabbits during the breeding season. Kolmer ('10) who studied the histology of the suprarenal glands of guinea-pigs, shows clearly an intimate relation between the sex glands and the suprarenals. He states that there exist definite cyclical changes of the suprarenals following corresponding changes of the sex glands in functional activity.

It is evident from this that the suprarenal glands are intimately related to the sex glands, both in males and in females. My own investigations also show clearly such relations between the two sets of glands in the rats; i.e., the suprarenal glands exhibit characteristic alterations after gonadectomy. It has been found that the suprarenal glands of the castrated rats exhibit an increase of 8.5 per cent to 17.6 per cent on the average, while those of the spayed rats show a decrease of 5.3 per cent to 25.0 per cent compared with their respective controls. These variations in the reaction in the two sexes are very regular and occur in all series and in most groups in each series. Why the suprarenals

respond differently in the two sexes as the result of gonadectomy cannot be explained at present, and we therefore merely note this singular phenomenon. There are no other corresponding observations on rats in the literature, so that our results must be compared with those obtained from other animals.

It is interesting to note, as previously stated, that Soli ('09) records a reduction of the suprarenals as the result of castration, while Marrassini and Luciani ('11) using the same species of animals, noted an increase. These diametrically opposed results may mean that the individual variation of the suprarenals is greater than that produced by castration, or that the suprarenals may not respond similarly at different seasons, or in the presence of different diets. As will be seen later, these investigators obtained after castration opposite results for the size of the hypophysis also. We must therefore await the results of further experiments with a much larger number of animals belonging to the species studied, maintained under very definite nutritive conditions, before endeavoring to reach a conclusion.

In this connection it is important to point out once more that the size of the suprarenal glands in rats differs strikingly according to sex (Hatai '13). The female rats have considerably larger glands than the males. This sexual difference is evident prior to sexual maturity. Gonadectomy in the male increases the weight of the suprarenals at the same time that gonadectomy in the female reduces the weight, thus bringing the two sexes nearer together in this character. Moreover, we notice in several other instances after gonadectomy, the similar phenomenon of the approach of characters which are differently modified in the normal rat according to sex. The weights of the suprarenals are not altered to any noticeable extent by partial gonadectomy, either in the males or the females.

Thymus. The effect of gonadectomy on the size of the thymus has been studied by numerous investigators. Calzolari ('98) found the thymus in the castrated rabbits strikingly enlarged. He considers that the normal atrophy of the thymus is delayed by the removal of the testes. Henderson ('04) obtained similar results in rabbits, guinea-pigs and cattle. Goodall ('04) noted

also an enlarged thymus in castrated guinea-pigs. Hammer ('05) and Tandler and Grosz ('07) found the thymus enlarged in eunuchs. Further, Soli found the same ('09) in chicks and rabbits; Marrassini ('10) in chickens, and Gellin ('10) in both sexes of rabbits obtained similar results. Still other investigators have noted the hypertrophy of the thymus after gonadectomy and their papers are cited in the works of Biedl ('13) and of Vincent ('12), to which the reader is referred.

It is clear, therefore, that all the investigators agree that gonadectomy increases the size of the thymus in both sexes. As can be seen from table (20), my own investigations on albino rats are in full accord with this result. The weight of the thymus after gonadectomy is almost twice that in the control rats, and furthermore the enlargement occurs in all the series with the exception of the semi-castration series at 17-27 days in which series the reaction is anomalous. We further note that gonadectomy in young animals not only enlarges the thymus and delays its normal involutionary process, but that the gland responds even after it has atrophied to a considerable extent. The experiments upon the rats whose ages were about 200 days show this clearly (pp. 9 and 17). Although we have not examined histologically the normal thymus at 200 days, nevertheless its gross appearance shows that much of the mass is represented by connective tissue. Yet even in such a highly atrophic stage, the thymus can respond nearly as well as when gonadectomy has been performed at a much earlier age.

Hypophysis. The alterations in the size of the hypophysis following gonadectomy have been much studied and we are therefore in a position to discuss these alterations somewhat fully.

Fichera ('05) has shown that the removal of the testes in fowl and cattle enlarges the hypophysis. He also has shown that spaying produces similar enlargement of the hypophysis in rabbits and guinea-pigs. Fichera considers this hypertrophy of the hypophysis as a physiological compensation, supplementing the loss of the sex glands. Fichera's observations were confirmed by numerous investigators using not only the same species, but also several other kinds of animals. We shall cite only a few of

the investigators whose work seems most closely related to our own, and for the full bibliography refer the reader to the works of Biedl ('13) and Vincent ('12). Enlargement of the hypophysis has been reported by Parhon and Goldstein ('05) in spayed rabbits and dogs; by Cimoroni ('08) in castrated rabbits and dogs; by Kon ('09) and by Tandler and Grosz ('10) in both men and women; by Hatai ('12) in castrated rats; by Zacherl (cited by Biedl '13) in both male and female rats; and by Soli ('09) in castrated fowls and rabbits. Livingston ('14) finds an enlarged hypophysis in spayed rabbits, but not in castrated. Lastly, this present experiment gives again an instance of enlarged hypophysis in the male albino rat after castration (table 2, 3 and 4) while spaying also is followed by an increase, which, however, is only slight.

At the same time, there is an almost equally numerous group of investigators who have not found an enlargement of the hypophysis after gonadectomy. For instance, Pirsche ('02) did not obtain any enlargement of the hypophysis from castrated guinea-pigs, but at the same time he found a noticeable increase in the body weight. At autopsy he did not notice any unusual deposition of fat. Barnabo ('08) failed to find an enlargement of the hypophysis in castrated albino rats, though he found an enlargement in a rat, one testis of which had been removed and the vas deferens of the other testis ligated. Marrassini and Luciani ('11) using fowls, cattle, guinea-pigs and rabbits (the kinds of animals used by Fichera) failed to notice an hypertrophy of hypophysis, though they noted the enlarged suprarenal glands. Hatai ('13) noticed in the spayed albino rats an increase of the hypophysis which was small as compared with that following castration. Livingston ('14) in a large number of rabbits failed to find an enlargement of the hypophysis in the castrated males, but noted an overgrowth of the body in weight. In the spayed female rabbits, however, Livingston found a noticeable degree of hypertrophy of the hypophysis, but failed to obtain an overgrowth in body weight. Lastly, my own present experiment fully confirms the findings of the previous experiment: that is, in the castrated rats the hypophysis enlarges very strikingly, while the

growth of the body is nearly normal. On the other hand, in the spayed rats the hypophysis shows only an insignificant increase, but this is accompanied by greater growth, both in body weight and in body length.

I have mentioned already the experiments of Barnabo and of Zacherl, both of whom used albino rats. Since my own observations are based entirely on gonadectomized albino rats, and since the foregoing is the only work with which our own can be directly compared, it will be worth while to review in some detail the results obtained by these two investigators.

Barnabo's ('08) experiments were as follows:

Series 1. Bilateral section of vas deferens. This series comprises three rats.

Series 2. Section of vas deferens on one side and castration on other. This series comprises two rats.

Series 3. Bilateral castration. This series comprises three rats.

Barnabo's operations were performed on sexually mature rats (112 grams in average body weight) and the period between operation and death was on the average 76 days. As long as we have made no experiments corresponding to Barnabo's Series 1 and 2, we shall pass them by and consider only the results obtained from his Series 3. There we find the average weight of the hypophysis as reported by him was 0.015 grams in rats with a final body weight of 151 grams. Barnabo concludes that the weight of the hypophysis is not altered as the result of castration. No data are given regarding the control rats, and it is merely stated "*Per altro controllo mi sono poi servito di animali normali.*" However, according to our formula (Hatai '13) the weight of the hypophysis given by Barnabo is almost 150 per cent heavier than it ought to be for the body weight given. For this peculiar result no satisfactory explanation can be given, but I venture to suggest that it is just possible that Barnabo did not realize the existence of the striking sex difference in the weight of the hypophysis, and by an oversight compared the weight of the hypophysis in the castrated rats with that in normal females, in which case but slight difference might appear, as at

this period the weight of the hypophysis in the female is more than twice that in the male (Hatai '13). In any case, the number of rats used by Barnabo is certainly too small to furnish the basis for any definite conclusion.

Concerning Zacherl's work, we have too little information for criticism. The following is Biedl's ('13) statement concerning Zacherl's work: "Bei Ratten tritt nach der Kastration, wie aus den Versuchen, welche Dr. Zacherl in unserem Institute ausgeführt hat, hervorgeht, konstant eine Volumzunahme der Hypophyse ein, u. zw. sowohl bei männlichen, wie bei weiblichen Tieren." Nothing is given regarding the number of rats used for either control or operation and no numerical data concerning the weight of the hypophysis are given, so that the extent of the alteration cannot be determined.

So far as I am aware, these are all the statements we have regarding the effect of gonadectomy in the albino rat on the weight of the hypophysis, and for reasons already given, we must await further work before explaining the differences between the results of Barnabo on the one hand and those of Zacherl and ourselves on the other.

The brief review of the literature given above reveals the fact that the findings as to the effect of gonadectomy on the size of the hypophysis are diametrically opposed to each other. Probably the contradictory results obtained may be traced to one or more of three causes:

- (1) Insufficiency in the number of animals used.
- (2) Variations in the weight of the hypophysis arising from confusion of the sexes or from the use of different litters, or still more from the use of different strains of the same race.
- (3) True variations in the response of the hypophysis associated with differences in the response of the body to gonadectomy.

The first two suggestions have been discussed in my previous paper (Hatai '13) and I will therefore limit my comments here to the third cause named.

It is highly probable that the hypophysis may not respond always in the same manner, but vary in its response according to the condition of some other parts of the body. For instance,

I have often noted that some spayed rats presented a considerable increase in the weight of the hypophysis when these animals failed to show any overgrowth and obesity. The reaction of the hypophysis in this instance is similar to that in the castrated rats. Similarly, the castrated rats give a very slight or practically no increase in the weight of the hypophysis, when these show, as they sometimes do, a marked obesity. In this case the reaction of the hypophysis is much the same as that of the typical spayed rats. Such instances are not at all infrequent. In fact, in the series here described all the female rats whose ovaries were removed gave much greater increase in the weight of the hypophysis than those belonging to the previous series (Hatai '13). The difference in the response in the two series is explained by the fact that the spayed rats belonging to the earlier experiment (1912 series) showed considerable obesity, while those belonging to the present series did not, although the body had gained both in weight and in length. It seems therefore probable that the size of the hypophysis and body changes, especially fat deposition, are inversely related.

The evidence just given suggests that the contradictory findings by various observers may be due in part to these differences in response shown by the body as a whole, or by some of the other ductless glands. It is probable therefore that if the three conditions mentioned above were carefully regarded we might in the future obtain more harmonious results. Since this survey indicates that the weight of the hypophysis and of the body are reciprocally related, I wish to show how far this suggestion may be used to explain some discrepancies already found in the literature.

We have noted that Pirsche ('02) found an overgrowth of the body in weight in the castrated guinea pigs, but did not find the enlargement of the hypophysis. Pirsche's observation is certainly contrary to the observation of Fichera ('05) who found an enlarged hypophysis, but possibly the discrepancy may be traced to the different reactions of the body in weight. Unfortunately Fichera does not give us any information regarding the growth of the body and thus we are unable to test the point in this case.

However, Livingston's ('14) experiments on rabbits demonstrate the reciprocal relation in the weight of the hypophysis and of the body, and thus it appears probable that different results obtained by others who also used rabbits, may be traced to the differences in the response of the body. There seems to be analogous variations in the case of the other ductless glands. Paton ('13) gives the following instance of a typical response of the testes after thymectomy in guinea-pigs:

When studying the action of testes and thymus on growth, I found that one guinea pig in my series, after removal of the thymus, hardly grew at all, although remaining quite well and active. When it died at over four months old, it was like an animal about a fortnight old, and the testes were in size and structure those of an animal of the same age. They had undergone no compensatory hypertrophy, as they usually do, and the result had apparently been arrest of growth which occurs when thymus and testes are removed together.

I have shown elsewhere (Hatai '13) that in albino rats the hypophysis of the female is more than twice as heavy as that of the male. This sexual difference is evident prior to sexual maturity. Our present observations show this sexual difference to exist in the norway rats, although it is slight in amount. Whether or no a sexual difference exists in other mammals has still to be determined. Whatever may be the true function of the hypophysis and its relation to the body, we have interesting quantitative relations between the size of the hypophysis and size of the body. The clinical as well as the experimental evidence (Cushing '09, and Vincent '12) shows with a high degree of probability that 'hyposecretion' of the hypophysis produces an abnormal deposit of fat and a tendency to gigantism. Thus if a compensatory growth of the hypophysis does not follow, as is the case after spaying, the product of the unaltered gland must be employed for two purposes, one to replace the ovarian hormone, and two, for the normal uses, whatever these may be. Spaying thus appears to overtax the unmodified gland, and consequently to produce phenomena similar to those following hyposecretion. On the other hand, compensatory hypertrophy of the hypophysis in the male albino rats after castration appears

to prevent the phenomena of hyposecretion, and consequently the body changes are absent. The typical cases mentioned above may be interpreted on the same principle. The results of semi-spaying and of semi-castration in the albino rat gives still further evidence in support of the view just presented. Partial gonadectomy produces neither an enlargement of the hypophysis, nor abnormal fat deposition in the body. This is explained by the fact that the remaining sex gland undergoes a compensatory hypertrophy, and thus the normal secretory function is performed adequately.

Ovaries. A compensatory hypertrophy of the surviving ovary after semi-spaying has been noted by several investigators. Bond ('06), Carmichael and Marshall ('08) and Fichera ('10) all reported this in semi-spayed rabbits. In my previous paper (Hatai '13) I have also shown the compensatory hypertrophy of the remaining ovary in the semi-spayed albino rat. The present investigation confirms the previous results, and indeed the semi-spayed rats of the present series give a surviving ovary of more than twice the normal size. This compensatory growth of the ovary evidently prevents all body changes after semi-spaying, as I have not been able to find any noticeable alterations in the other characters examined. I have not made any histological examination to determine exactly what tissue is responsible for this enlargement of the ovary. However it is probable that this increase is caused by the overgrowth of the interstitial cells rather than of the germinal or follicular cells. This statement is based on the results of transplanting ovaries into spayed animals. It has been noted by numerous investigators that when the ovaries are thus transplanted all the structures except the interstitial cells undergo degenerative changes, yet even when so modified, the transplanted ovary is capable of preventing the typical reactions of ovariectomy. Steinach ('12) demonstrated that the interstitial tissue alone is sufficient to produce feminine traits in the castrated albino rats to which the ovaries were transplanted.

Testes. So far as I am aware, an enlargement of the surviving testis following semi-castration has not yet been reported.

Failure to observe this response is probably due to the lack of good reference data such as we possess in our laboratory records, and which are necessary to reveal such a change. It has been stated that the testis of the semi-castrated rat shows an increase of 14 to 15 per cent. This increase though small, is highly uniform and occurs in all the series.

I have shown that in the case of the semi-spayed rats the compensatory hypertrophy of the surviving ovary is nearly perfect, and that probably it is the interstitial tissue which increases. Let us see whether the same reaction is possibly occurring in the case of the testes.

Bouin and Ancel ('03 and '04) have brought evidence to show that it is the interstitial cells which are essential for inhibiting the characteristic alterations after castration. They removed one testis in the rabbit and ligated the vas deferens of the other. After ten to twelve months they found a marked hypertrophy of the interstitial cells. They found also that some rabbits, after ligation of the vas deferens, developed characters which follow castration, and they believed that this occurred when nerve as well as the duct was ligated. Vincent and Coleman (cited by Paton '13) found that ligation of the whole spermatic cord, with blood vessels, acts in the same way as castration. On ligating the spermatic cord in rats, I have found the phenomena similar to those reported by Vincent and Coleman. When after this operation the entire testes, including the interstitial cells, degenerate, then the animal manifests the castration reaction. However, if the testes manage to survive partially, though all the germinal cells are degenerated, the rats do not exhibit the castration reaction. From this it seems reasonable to infer that the increase of the remaining testis after the removal of the other must be due to hyperplasia of the interstitial cells. If this inference is correct, the increase of the interstitial tissue though absolutely slight in comparison with the entire testes (for it is only a small fraction of the normal testes), should be relatively large when referred to itself as a standard. Unfortunately we have no data for the rat bearing directly on the normal amount of interstitial tissue in the testes. There are,

however, some observations on man. Hofmeister ('72) estimates in the human testes at the fourth month the amount of the interstitial tissue to be two-thirds of the whole testes. At eight years of age it constitutes only one-tenth of the organ, but at puberty it again increases somewhat. Taking Hofmeister's estimate of one-tenth of the whole testis for the interstitial tissue as applicable to the adult albino rat, we find the following interesting relations: From table 12 it appears that the average weight of both testes is 2.329 grams for body length of 217 mm. in the control rat, and the weight of the single testis of the semi-castrated is 1.239 grams for a body length of 209 mm. Since the weight of the testes increases with increasing body length, the weight of the testes of the control and of the experimented cannot be directly compared until the difference for the body lengths in the two forms is adjusted. When this correction is made according to the formula (Hatai '13) we find that the one testis of the control is 14 per cent less in weight than the single testis of the semi-castrated. We have assumed that the amount of the interstitial tissue is one tenth of the whole testis, accordingly we find the interstitial tissue to be 0.117 grams for the control and 0.124 grams for the semi-castrated. To this latter value 14 per cent of the weight of the single testis of the control ($= .163$) must be added to give the assumed value of the interstitial tissue of the semi-castrated. We thus obtain 0.287 grams of interstitial tissue for the single testis of the semi-castrated against 0.117 grams for one testis or 0.233 for both testes of the controls. Thus if we apply the increase of 14 per cent in the entire test is to the interstitial tissue alone, the increase of this latter amounts to over 100 per cent above that of the control. This result depends of course entirely on the correctness of assumption that one-tenth of the whole testes in the albino rats consists of interstitial tissue. Although direct proof of this is lacking, the assumption seems to have some evidence in its favor and none against it. Thus the increase of 14 per cent in the testis as a whole after semi-castration is misleading, for in all probability it really means a doubling in the amount of the interstitial tissue;

a response almost as complete as that shown by the ovary after semi-spaying.

Skeletal system. Alterations of the bones in weight and in length after gonadectomy have been reported by a number of investigators. Poncet ('97) reported upon rabbits in which he found that castration produces stronger and especially longer bones. Pirsche ('02) found also stronger and longer bones in castrated guinea-pigs. Sellheim ('99) found similar phenomena in the castrated dog. With eunuchs Tandler and Grosz ('13) found constantly longer bones. Without giving any further evidence, it is safe to say that gonadectomy according to these investigators causes an abnormal elongation, and occasionally a thickening of the bones.

In my experiments on albino rats, I have found that the ratio between body weight and bone weight and the ratio between body length and bone length tend to be slightly higher in the gonadectomized rats than in the controls. The alteration, though constant, is certainly very slight, and indeed the difference between the control and experimented becomes evident only after careful computation. On the other hand, one gets the impression that the skeletal changes reported by other investigators were so large that they could be detected at a glance.

Corresponding data on rats obtained by other investigators are not available for comparison, and thus I am unable to say whether the slight alteration of the bones in weight and length are to be regarded as typical for the albino rat after gonadectomy. However, the water content of the bones was distinctly higher in the gonadectomized rats. At the present moment the interpretation of this phenomenon is not clear. In the semi-castrated rats, there was no alteration either in length, weight or in the amount of water in the bones.

In this connection it may be interesting to note that the tail length of the castrated is noticeably longer than that of the control rat. The difference amounts to 3.2 per cent to 4.7 per cent in the rats which have been operated on at 19 days and 86 days respectively. The rats castrated at 220 days gave a dif-

ference of 1.8 per cent. On the other hand, the spayed rats do not give this response. To interpret this response will require further experiment, but in the mean time the fact of this occurrence is worthy of note.

Central nervous system

Gonadectomy is apparently without influence on the central nervous system. The proportional weights of the different parts of the brain and the percentage of water in the several divisions of the system are unmodified, while the slight alterations in the absolute weights of the brain and spinal cord are, by reason of their lack of uniformity, most readily explained as fluctuation. This result is one of prime importance from the standpoint of our general laboratory problem—the growth of the mammalian nervous system—for it shows that after 15 days of age, gonadectomy, complete or partial, is without influence on the gross structure or fundamental chemical make-up (water and solids) of the nervous system. Further, as the behavior of the female can be induced in the male by the implantation of ovarian tissue (Steinach '12) and the characteristic male behavior ceases after castration, we obtain good evidence for the view already put forward by Steinach that the respective gonads are activators of the nervous system according to sex, and in addition we now have evidence that this is accomplished without recognizable changes in either the structure or the fundamental make-up of the system.

Secondary sexual characters

Recently Steinach ('10, '12) has made a careful study of these in the albino rat and noted modifications in size of the secondary sex glands, nipples and bones, the changes in hair and in behavior. To the list of modifiable characters which Steinach has given, I am able to make the following additions: The hypophysis of the normal male rat is considerably smaller than that of the female. Castration increases the weight of the hypophysis thus making it approach that of the female. The suprarenals of the male

are normally smaller than those of the females. Again castration increases the male suprarenals. At the same time, spaying reduces the suprarenals of the female, thus causing the two sexes to approach each other in this character. Further, we have found that the tail of the normal male is definitely shorter than that of the normal female. Castration increases the tail length of the male—thus approximating the relation in the female. Again, the body of the adult female is much smaller both in length and weight than that of the adult male. Spaying induces an overgrowth of the female body both in length and in weight, while castration tends to make the male body somewhat less than normal, thus bringing the two sexes nearer together. Speaking very generally, we might say that gonadectomy tends to produce the secondary characters found in the opposite sex.

SUMMARY

All the experiments were made on albino rats. There were five series, representing five different operations:

1. Total gonadectomy: castration and spaying.
2. Partial gonadectomy: semi-castration and semi-spaying.
3. Ligation of spermatic cord.
4. Removal of one ovary, followed by an isolation of the other ovary from the uterus.
5. The isolation of both ovaries from the uterus.

The following body characters and organs (or some of them) were measured in each series: Body length, body weight, tail length, sex glands, thyroid, suprarenals, thymus, hypophysis, various long bones and the central nervous system. The main results are given under the characters studied.

(1) *Body length.* Comparing the operated animals with the controls, the absolute body lengths were found to be slightly less in all the operated rats except the spayed females in which the body lengths were distinctly greater.

(2) *Tail length.* The tail with respect to the body length tends to be slightly longer in the castrated males, but no significant alterations occur in the other series.

(3) *Body weight.* The body weight in respect to body length is greater in nearly all operated rats, but especially is this the case in the spayed rats.

(4) *Weight and length of the bones.* These characters are not modified in the semi-spayed rat, but in the castrated as well as in spayed rats, the bones (femur, tibia and fibula; humerus, radius and ulna) tend to be very slightly longer and heavier than in the corresponding controls. The bones were not examined in the other series. The percentage of water in the bones is also slightly higher in the operated than in the controls. No alteration was noted in the semi-spayed in this respect.

(5) *Central nervous system.* No characteristic response can be reported for the central nervous system. The differences in total weight after gonadectomy appear to be fluctuations merely. The different parts of the brain were not modified in their relative weights, and the water content of the brain and of the spinal cord were the same in both the operated and in the controls.

(6) *Sex glands.* In the semi-spayed series the compensatory growth of the remaining ovary is almost perfect as it attains nearly twice its normal size. In the case of the semi-castrated series the remaining testis shows an increase of 14 per cent as a whole. If however we assume that the increase is due solely to hyperplasia of the interstitial tissue, and further, if we take Hofmeister's estimate of the interstitial tissue as one tenth of the entire testes in the human testes and apply it to the rat, we obtain compensatory growth in the interstitial tissue in the semi-castrated rat which is almost as great as in the case of the remaining ovary of the semi-spayed rat. The isolated ovaries survived and grew as if these had been connected with the uterus. In the case of isolation of the ovary followed by semi-spaying, the remaining isolated ovary hypertrophies in the same manner as that of the semi-spayed rat. The ligation of the spermatic cord in certain cases causes a complete atrophy of the testes, thus producing the alterations of the body characters similar to those which appear in the castrated rats.

(7) *Thyroid gland.* On account of its great variability in weight, no definite conclusions can be drawn from the variations observed in the weight of the thyroid gland.

(8) *Suprarenal glands.* The suprarenal glands show opposite reactions in the two sexes as the result of gonadectomy. In the castrates, the suprarenals show an increase, and in the spayed rats, a decrease. No adequate explanation can be given for this singular phenomenon. The suprarenal glands fail to give any response in all the other experiments, except those where the spermatic cords were ligated. In these last the suprarenals show reactions similar to those which follow castration in group II where the testes has been absorbed.

(9) *Thymus.* The thymus gland shows a very striking response to gonadectomy, though no noticeable alterations were found to follow partial gonadectomy. After the former operation the thymus increases to almost twice the size which it has in the control rats. The thymus seems not only to delay its normal involutionary process but actually to increase in weight as the result of gonadectomy. Gonadectomy enables the thymus of the old rat to respond, and thus a highly atrophied thymus may become enlarged almost as much as if the operation were performed on young animals.

(10) *Hypophysis.* Following the removal of the testes, the weight of the hypophysis is increased on the average as much as 50 per cent. On the other hand, spaying produced only a slight increase (about 8 per cent on the average). This difference in reaction according to sex has been noted in my previous experiments (1912 series). All the other series show an almost insignificant variation, except the ligation of the spermatic cord which, when followed by absorption of the testis, gives a response similar to castration.

(11) *Relations of the hypophysis to the growth of gonadectomized rats.* After removal of the sex glands, it appears that when compensatory growth of the hypophysis occurs, there is no overgrowth of the body or obesity. On the other hand, both these latter responses appear when the enlargement does not occur—as

in the spayed rats, for example. In the semi-spayed, as well as in the semi-castrated, neither enlargement of the hypophysis nor overgrowth and obesity occur, because the enlargement of the remaining ovary or testis enables the surviving sex gland to furnish the normal amount of gonadine.

(12) *Secondary sexual characters.* The total removal of the sex glands tends to increase the resemblance between the two sexes, or, in other words, to reduce the differences in those secondary characters which, in the normal animal, are marks of sex. Put in another way, we may say with equal truth that gonadectomy in a given sex tends to produce the secondary characters found in the opposite sex.

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TABLE 1

Giving the method of computing the percentage deviation by the use of the formulas

GROUP VII—CASTRATION WEIGHT IN GRAMS LENGTH IN MILLIMETERS	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	THYROID	SUPRA- RENALS	THYMUS	HYPOPHY- SIS	CENTRAL NERVOUS SYSTEM		PERCENTAGE OF WATER		AGE DAYS
								Brain	Sp. cord	Brain	Sp. cord	
Controls; observed values	212	180	229.5	0.0408	0.0355	0.2139	0.0080	1.925	0.575	78.382 ¹	71.337 ¹	150
Controls; values calculated from formulas (body length taken as basis of computation)												
A. Percentage deviation of observed from calculated values	212	180	238.1	0.0369	0.0382	0.2106 ¹	0.0086	1.911	0.611			
Operated; observed values	0	0	-3.6	10.6	-7.1	15.8	-7.0	0.73	-5.9			
Operated; values calculated from formulas (body length taken as basis of computation)	205	181	217.6	0.0369	0.0389	0.2280	0.0118	1.943	0.578	78.535 ¹	72.186 ¹	150
B. Percentage deviation of observed from calculated values	205	174	211.4	0.0336	0.0354	0.2106 ¹	0.0078	1.880	0.583			
Amounts by which the percentage for the operated animals differ from those for the controls, i.e., B-A	0	4.0	2.9	9.8	9.9	103.2	51.3	3.4	-0.86			
	0	4.0	6.5	-0.8	17.0	87.4	58.3	2.6	5.0	0.15	0.85	

¹ Values according to age.

TABLE 2

Giving the weight (grams) and length (mm.) data for the castrated albino rats compared with those for the controls. After castration at 16 to 22 days

GROUP		NO. CASES		TAIL LENGTH	BODY WEIGHT	THYROID	SUPRA-RENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYSTEM		PER CENT WATER		NO. DAYS AFTER OPER.	AGE DAYS
		BODY LENGTH								Brain	Sp. Cd.	Brain	Sp. Cd.		
V	Control.....	3	199	170	216.3	0.0835	0.0271	0.1600	0.0073	1.679	0.49479	0.5872	114	122	
	Operated.....	3	196	174	203.0	0.0533	0.0330	0.2723	0.0104	1.745	0.50579	0.5972	490	100	122
	Per cent: Oper.—Cont.....		0	4.3	-1.3	-90.3	20.9	63.5	49.3	4.3	3.9	0.10	0.38		
	Control.....	5	206	174	203.8	0.0344	0.0392	0.1530	0.0082	1.909	0.56278	0.4791	404	144	
VI	Operated.....	4	200	173	202.2	0.0381	0.0435	0.3299	0.0116	1.944	0.56878	0.48371	504	128	144
	Per cent: Oper.—Cont.....		0	2.9	9.4	20.1	20.4	73.2	55.1	3.4	5.0	0.00	0.10		
	Control.....	7	212	180	229.5	0.0408	0.0355	0.2439	0.0080	1.925	0.57578	0.38271	337	150	
	Operated.....	8	205	181	217.6	0.0369	0.0389	0.4280	0.0118	1.943	0.57878	0.53572	186	130	150
VII	Per cent: Oper.—Cont.....		0	4.0	6.6	-0.8	17.02	87.4	58.3	2.6	5.0	0.15	0.85		
	Control.....	5	221	183	263.4	0.0459	0.0406	0.1895	0.0090	1.950	0.64378	0.35571	242	163	
	Operated.....	6	209	183	228.4	0.0365	0.0393	0.3195	0.0131	1.891	0.58278	0.35071	380	146	163
	Per cent: Oper.—Cont.....		0	6.0	5.9	-7.7	12.9	65.8	67.0	0.37	-2.4	-0.01	0.14		
IX	Control.....	4	213	182	259.0	0.0692	0.0326	0.1573	0.0087	1.760	0.58679	0.3872	101	167	
	Operated.....	3	208	186	255.9	0.0574	0.0353	0.3005	0.0134	1.817	0.59078	0.92571	669	149	167
	Per cent: Oper.—Cont.....		0	4.5	8.1	-21.0	12.8	73.9	65.4	4.1	3.9	-0.11	-0.43		
	Control.....	3	227	188	319.6	0.0788	0.0304	0.2008	0.0129	1.940	0.64378	0.60170	728	193	
X	Operated.....	2	208	183	248.2	0.0458	0.0324	0.2062	0.0136	1.887	0.59378	0.54670	913	173	193
	Per cent: Oper.—Cont.....		0	6.5	7.2	-44.6	21.4	3.2	45.0	0.92	4.2	-0.55	0.19		
	Control.....	27	213	180	248.6	0.0588	0.0344	0.1841	0.0090	1.861	0.58478	0.65271	488	157	
	Operated.....	26	204	180	225.9	0.0447	0.0371	0.3094	0.0123	1.871	0.57078	0.66671	690	138	157
V-X	Per cent: Oper.—Cont.....		0	4.7	6.0	-21.5	17.6	61.2	56.7	2.6	3.3	0.01	0.20		

TABLE 3

Giving the weight (grams) and length (mm.) data for the castrated albino rats compared with those for the controls. After castration at 79 to 93 days

GROUP		NO. CASES	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	THYROID		SUPRA-RENALS		THYMUS		HYPOPHYSIS		CENTRAL NERVOUS SYSTEM		PER CENT WATER		NO. DAYS AFTER OPER.	AGE DAYS
														Brain	Sp. Cd.	Brain	Sp. Cd.		
XI	Control	4	224	182	286.7	0.0577	0.0495	0.1476	0.0092	1.976	0.66978	18670	240	191					
	Operated	3	208	177	222.9	0.0388	0.0382	0.2278	0.0124	1.889	0.62078	07070	773	98	191				
	Per cent; Oper.—Cont.....		0	5.2	2.1	—22.7	—5.9	73.0	62.0	—0.40	2.5	—0.12	0.53						
XII	Control	2	197	164	190.5	0.0300	0.0346	0.0978	0.0075	1.756	0.57078	40070	852	202					
	Operated	2	206	175	230.5	0.0442	0.0452	0.2221	0.0103	1.822	0.59578	17170	892	123	202				
	Per cent; Oper.—Cont.....		0	1.2	3.9	30.3	19.5	76.8	23.2	1.4	—1.9	—0.23	0.04						
XI XII }	Control	6	211	173	238.6	0.0439	0.0421	0.1227	0.0084	1.866	0.62078	29370	546	197					
	Operated	5	207	176	226.7	0.0415	0.0417	0.2250	0.0114	1.856	0.60878	12170	833	111	197				
	Per cent; Oper.—Cont.....		0	3.2	3.0	3.8	6.8	74.9	42.6	0.51	0.32	—0.17	0.29						

TABLE 4

Giving the weight (grams) and length (mm.) data for the castrated albino rats compared with those for the controls. After castration at 208 to 232 days

GROUP		NO. CASES	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	THYROID		SUPRA-RENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYSTEM		PER CENT WATER		NO. DAYS AFTER OPER.	AGE DAYS
						Brain	Sp. Cd.				Brain	Sp. Cd.				
XIII	Control.....	3	217	177	248.2	0.0636	0.0355	0.0757	0.0087	1.901	0.61978	18370.563		259		
	Operated.....	4	213	178	226.6	0.0665	0.0386	0.0979	0.0106	1.843	0.59578	19370.324		44	259	
	Per cent: Oper.—Cont.....		0	2.7	-2.2	6.4	11.9	19.1	27.3	-2.2	-1.5	0.01	-0.24			
	Control.....	2	232	184	305.3	0.0559	0.0304	0.1339	0.0109	1.899	0.64377	95269.543		268		
XIV	Operated.....	2	224	183	294.4	0.0453	0.0365	0.2542	0.0157	1.909	0.63178	29370.296		60	267	
	Per cent: Oper.—Cont.....		0	3.4	9.4	-12.0	20.0	109.7	58.1	2.2	2.6	0.34	0.75			
	Control.....	2	222	190	268.0	0.0539	0.0378	0.0578	0.0104	1.994	0.67777	82069.760		292		
	Operated.....	2	217	184	270.1	0.0448	0.0335	0.0886	0.0127	1.924	0.65177	77969.857		60	292	
XV	Per cent: Oper.—Cont.....		0	-0.54	9.1	-14.3	-5.4	32.8	31.9	-2.4	-0.82	-0.04	0.10			
	Control.....	7	224	184	273.8	0.0578	0.0346	0.0858	0.0100	1.931	0.64677	98569.955		273		
	Operated.....	8	218	182	263.7	0.0522	0.0362	0.1469	0.0130	1.892	0.62678	08570.159		55	273	
	Per cent: Oper.—Cont.....		0	1.8	5.4	-6.7	8.5	53.9	39.1	-0.74	0.08	-0.10	0.19			

TABLE 5

Percentage weight of the parts of the encephalon in albino rats castrated at 16-22 days

	BODY LENGTH	BRAIN	CEREBRUM	STEM	CEREBELLUM	OLF. BULBS	NO.	AGE
A.	Control.....	1.929	1.221	0.358	0.281	0.069	5	119
	Per cent.....	100.0	63.3	18.6	14.6	3.8		
	Operated.....	1.890	1.208	0.351	0.274	0.057		
B.	Control.....	1.955	1.229	0.369	0.278	3.7	5	147
	Per cent.....	100.0	62.9	18.9	14.2	4.0		
	Operated.....	1.909	1.208	0.361	0.278	0.062		
C.	Control.....	1.888	1.161	0.368	0.282	3.2	3	161
	Per cent.....	100.0	61.6	19.5	15.0	4.0		
	Operated.....	1.917	1.198	0.374	0.276	0.069		
D.	Control.....	1.819	1.108	0.360	0.274	3.6	4	168
	Per cent.....	100.0	60.9	19.8	15.0	4.3		
	Operated.....	1.816	1.123	0.355	0.272	0.066		
E.	Control.....	1.992	1.245	0.390	0.277	3.7	2	173
	Per cent.....	100.0	62.6	19.6	13.9	4.0		
	Operated.....	1.887	1.185	0.380	0.265	0.057		
F.	Control.....	1.787	1.093	0.378	0.278	3.0	3	213
	Per cent.....	100.0	61.2	21.2	15.6	2.1		
	Operated.....	1.837	1.144	0.370	0.277	0.046		
A-F	Control.....	1.805	1.176	0.371	0.278	2.5	22	164
	Per cent.....	100.0	62.1	19.6	14.7	3.7		
	Operated.....	1.876	1.178	0.365	0.274	0.060		
	Per cent.....	100.0	62.8	19.5	14.6	3.3	22	164

TABLE 6

The weights (grams) and lengths (mm.) of the bones together with the percentage of water in them in the castrated albino rats.

	BODY (1) WEIGHT (2) LENGTH	HUMERUS		RADIUS AND ULNA		FEMUR		TIBIA AND FIBULA		ALL BONES	AGE
		Right	Left	Right	Left	Right	Left	Right	Left		
Control (2) No. 24b	{ Fresh weight.....	0.289	0.289	0.192	0.192	0.654	0.651	0.534	0.541	3.342	115
	{ Dried weight.....	0.210	0.212	0.164	0.169	0.465	0.458	0.388	0.388	2.454	
	{ Length.....	26.6	26.6			34.8	34.8			30.7	
Operated (3)	{ Fresh weight.....	0.228	0.224	0.155	0.150	0.508	0.505	0.416	0.409	2.595	
	{ Dried weight.....	0.162	0.160	0.129	0.127	0.344	0.340	0.297	0.295	1.854	
	{ Length.....	25.0	25.0			32.5	32.5			28.8	
Control (3) No. 25b	{ Fresh weight.....	0.264	0.268	0.170	0.168	0.559	0.596	0.460	0.461	2.946	122
	{ Dried weight.....	0.194	0.197	0.146	0.143	0.424	0.428	0.345	0.345	2.222	
	{ Length.....	26.5	26.5			34.1	34.3			30.4	
Operated (4)	{ Fresh weight.....	0.249	0.256	0.165	0.173	0.545	0.547	0.444	0.447	2.826	
	{ Dried weight.....	0.184	0.187	0.143	0.147	0.384	0.387	0.333	0.334	2.069	
	{ Length.....	26.0	26.0			33.3	33.3			29.7	
Control (3) No. 26b	{ Fresh weight.....	0.266	0.263	0.173	0.172	0.581	0.588	0.473	0.474	2.990	121
	{ Dried weight.....	0.196	0.195	0.148	0.147	0.416	0.422	0.356	0.359	2.239	
	{ Length.....	26.3	26.4			33.7	33.7			30.0	
Operated (2)	{ Fresh weight.....	0.229	0.231	0.153	0.149	0.470	0.482	0.397	0.392	2.503	
	{ Dried weight.....	0.170	0.169	0.131	0.129	0.332	0.343	0.300	0.294	1.868	
	{ Length.....	25.0	25.1			31.9	31.7			28.4	

TABLE 6—Continued

Control (1)	Fresh weight.....	326.4	0.345	0.350	0.207	0.204	0.776	0.804	0.612	0.600	3.898	162
	Dried weight.....		0.289	0.287	0.181	0.179	0.568	0.592	0.454	0.460	2.950	
	Length.....	236	28.8	28.9			37.1	37.1			33.0	
No. 45b												
Operated (1)	Fresh weight.....	202.5	0.246	0.248	0.151	0.155	0.547	0.534	0.401	0.422	2.704	
	Dried weight.....		0.170	0.174	0.129	0.131	0.352	0.344	0.298	0.308	1.906	
	Length.....	202	25.1	25.1			32.6	32.6			28.9	
Control (9)	Fresh weight.....	265.8	0.291	0.293	0.186	0.184	0.643	0.660	0.520	0.519	3.294	130
	Dried weight.....		0.215	0.216	0.160	0.160	0.408	0.475	0.386	0.388	2.466	
	Length.....	220	27.1	27.1			34.9	35.0			31.0	
Average												
Operated (10)	Fresh weight.....	206.2	0.238	0.240	0.156	0.157	0.518	0.517	0.415	0.418	2.657	
	Dried weight.....		0.172	0.173	0.133	0.134	0.353	0.354	0.307	0.308	1.932	
	Length.....	204	25.3	25.3			32.6	32.6			29.0	

TABLE 7

Giving the weight (grams) and length (mm.) data for the sprayed albino rats compared with those for the controls. After spraying at 19-30 days.

GROUP		NO. CASES	BODY LENGTH		TAIL LENGTH	BODY WEIGHT	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYSTEM		PER CENT WATER		NO. DAYS AFTER OPER.	AGE DAYS
											Brain	Sp. Cd.	Brain	Sp. Cd.		
VI	Control.....	7 183	160	155.6	0.0419	0.0491	0.1631	0.0089	1.602	0.45679.09472.264	126					
	Operated.....	7 191	171	202.8	0.0412	0.0440	0.3058	0.0105	1.668	0.49779.17472.627101126						
	Per cent: Oper.—Cont.....	0	2.4	13.2	-10.8	-24.5	60.6	0.37	1.8	2.4	0.05	0.36				
VII	Control.....	5 190	166	153.1	0.0287	0.0512	0.1192	0.0083	1.697	0.47578.44371.118	137					
	Operated.....	4 192	172	181.3	0.0337	0.0479	0.2900	0.0101	1.749	0.50878.35071.003107137						
	Per cent: Oper.—Cont.....	0	2.4	12.5	14.1	-10.2	69.6	11.7	2.4	4.7	-0.09	-0.12				
VIII	Control.....	4 190	163	176.4	0.0363	0.0515	0.1428	0.0090	1.679	0.48479.14972.963	144					
	Operated.....	3 199	171	221.6	0.0397	0.0461	0.2770	0.0110	1.737	0.52479.00672.200125144						
	Per cent: Oper.—Cont.....	0	-0.48	7.3	-4.0	-24.4	75.8	1.8	1.1	1.3	-0.14	-0.76				
XI	Control.....	5 197	166	199.9	0.0282	0.0435	0.1949	0.0088	1.776	0.52078.43971.349	161					
	Operated.....	5 198	176	210.1	0.0287	0.0412	0.2632	0.0120	1.848	0.54878.36971.603135161						
	Per cent: Oper.—Cont.....	0	5.2	3.3	0.46	-5.7	34.2	21.6	3.7	4.2	-0.07	0.25				
X	Control.....	5 203	176	211.8	0.0610	0.0487	0.1648	0.0123	1.759	0.53378.91872.077	162					
	Operated.....	5 213	189	257.5	0.0585	0.0399	0.3069	0.0150	1.804	0.59378.74472.000138162						
	Per cent: Oper.—Cont.....	0	1.7	2.2	-28.6	-25.2	71.5	0.25	0.23	3.7	-0.17	-0.08				
XI	Control.....	4 201	178	184.9	0.0488	0.0539	0.0982	0.0092	1.836	0.55578.34070.313	220					
	Operated.....	4 201	178	195.8	0.0650	0.0422	0.1543	0.0118	1.789	0.54978.74670.681190220						
	Per cent: Oper.—Cont.....	0	0	5.1	48.4	-20.9	38.3	17.6	-2.5	-1.0	0.41	0.37				
VI-XI	Control.....	30194	168	180.3	0.0408	0.0497	0.1422	0.0094	1.725	0.50478.73171.681	158					
	Operated.....	28199	176	211.5	0.0445	0.0436	0.2662	0.0117	1.766	0.53778.73271.386133158						
	Per cent: Oper.—Cont.....	0	1.9	7.3	3.3	-18.5	58.3	8.9	1.3	2.5	0.00	-0.30				

TABLE 8

Giving the weight (grams) and length (mm.) data for the spayed albino rats compared with those for the controls. After spaying at 97-119 days.

GROUP		NO. CASES	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYSTEM		PER CENT WATER		NO. DAYS AFTER OPER.	AGE DAYS
										Brain	Sp. Cd.	Brain	Sp. Cd.		
XII	Control	4	188	161	163.9	0.0167	0.0523	0.1385	0.0107	1.699	0.50978	47.271	170	198	
	Operated	3	188	156	181.1	0.0267	0.0394	0.1728	0.0108	1.728	0.52078	43.371	803	101	198
	Percent: Oper.—Cont.		0	-3.0	10.1	-66.9	-28.2	20.7	0.88	1.6	2.0	-0.04	0.63		
XIII	Control	5	199	174	192.1	0.0649	0.0519	0.1365	0.0132	1.820	0.54278	63.571	681	217	
	Operated	5	200	175	201.0	0.0536	0.0407	0.2235	0.0156	1.792	0.55178	52.571	807	98	217
	Percent: Oper.—Cont.		0	0	2.7	-34.4	-21.9	58.4	14.6	-1.6	0.90	-0.11	0.13		
XII-XIII	Control	9	194	168	178.0	0.0558	0.0521	0.1375	0.0120	1.760	0.52678	55.471	426	208	
	Operated	8	194	166	191.1	0.0402	0.0401	0.1982	0.0132	1.760	0.53678	47.971	805	100	208
	Percent: Oper.—Cont.		0	-1.5	6.4	-50.6	-25.0	29.6	7.8	0	1.5	-0.08	0.38		

TABLE 9

Giving the weight (gms.) and length (mm.) data for the spayed albino rats compared with those for the controls. After spaying at 172-195 days

GROUP		NO. OF CASES	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYS-TEM		PER CENT WATER		NO. OF DAYS AFTER OPER.	AGE DAYS
										Brain	Sp. Cd.	Brain	Sp. Cd.		
XIV	Control.....	5 184	159	155.1	0.0275	0.0383	0.1206	0.0082	1.765	0.51078	50971	660	211		
	Operated.....	3 184	161	150.5	0.0263	0.0341	0.1481	0.0075	1.708	0.49878	07571	074	27 211		
	Per cent: Oper.-cont.....	0	1.2	-2.9	-4.5	-9.8	17.9	-6.7	-3.2	-2.3	-0.43	-0.59			
XV	Control.....	2 195	166	158.9	0.0348	0.0398	0.1078	0.0074	1.789	0.53377	59369	536	221		
	Operated.....	2 188	166	148.6	0.0284	0.0359	0.1136	0.0100	1.752	0.51677	86270	314	36 221		
	Per cent: Oper.-cont.....	0	3.5	4.8	-11.5	0.52	4.0	31.2	-0.32	1.8	0.27	0.78			
XVI	Control.....	2 198	174	171.4	0.0414	0.0391	0.1289	0.0101	1.920	0.57077	61469	575	232		
	Operated.....	2 204	178	207.3	0.0651	0.0390	0.2120	0.0123	1.933	0.59977	81169	750	60 232		
	Per cent: Oper.-cont.....	0	-1.1	8.6	57.6	-6.8	60.8	6.7	-0.81	0.92	0.18	0.18			
XIV-XVI	Control.....	9 192	166	161.8	0.0346	0.0391	0.1191	0.0129	1.825	0.53877	90570	257	221		
	Operated.....	7 192	168	168.8	0.0399	0.0363	0.1579	0.0149	1.798	0.53877	91370	379	41 221		
	Per cent: Oper.-cont.....	0	1.2	3.5	13.9	-5.3	27.5	10.4	-1.5	0.16	0.01	0.13			

TABLE 10

Percentage weight of the parts of the encephalon in albino rats after spaying at 19-30 days

	BODY LENGTH	BRAIN	CEREBRUM	STEM	CEREBELLUM	OLF. BULBS	NO.	AGE
A	{ Control	1.701	1.073	0.325	0.251	0.052	5	148
	{ Per cent	100.0	63.1	19.1	14.8	3.0		
	{ Operated	1.756	1.111	0.343	0.253	0.049		
	{ Per cent	100.0	63.2	19.6	14.5	2.7		
B	{ Control	1.756	1.103	0.344	0.250	0.059	6	161
	{ Per cent	100.0	62.8	19.6	14.2	3.4		
	{ Operated	1.780	1.106	0.360	0.259	0.056		
	{ Per cent	100.0	62.1	20.2	14.6	3.1		
C	{ Control	1.716	1.056	0.333	0.254	0.073	4	163
	{ Per cent	100.0	61.6	19.4	14.8	4.2		
	{ Operated	1.762	1.098	0.349	0.261	0.054		
	{ Per cent	100.0	62.3	19.7	14.8	3.1		
D	{ Control	1.823	1.165	0.349	0.257	0.053	3	173
	{ Per cent	100.0	63.9	19.1	14.1	2.4		
	{ Operated	1.786	1.123	0.352	0.249	0.063		
	{ Per cent	100.0	62.9	19.7	13.9	3.5		
E	{ Control	1.736	1.081	0.340	0.254	0.062	5	216
	{ Per cent	100.0	62.3	19.6	14.6	3.5		
	{ Operated	1.706	1.074	0.335	0.253	0.041		
	{ Per cent	100.0	63.0	19.7	14.8	2.4		
A	{ Control	1.746	1.096	0.338	0.253	0.060	23	172
	{ Per cent	100.0	62.7	19.4	14.5	3.3		
	{ Operated	1.758	1.102	0.348	0.255	0.053		
	{ Per cent	100.0	62.7	19.8	14.5	3.0		
E	{ Control	1.746	1.096	0.338	0.253	0.060	23	172
	{ Per cent	100.0	62.7	19.4	14.5	3.3		
	{ Operated	1.758	1.102	0.348	0.255	0.053		
	{ Per cent	100.0	62.7	19.8	14.5	3.0		

TABLE 11

The weights (gms.) and lengths (mm.) of the bones together with the percentage of water in them in the spayed albino rats

	BODY (1) WEIGHT (2) LENGTH	HUMERUS		RADIUS AND ULNA		FEMUR		TIBIA AND FIBULA		AGE
		Right	Left	Right	Left	Right	Left	Right	Left	
Control (2)	Fresh weight.....	0.217	0.218	0.141	0.138	0.497	0.479	0.383	0.381	2 454
	Dried weight.....	0.162	0.163	0.124	0.121	0.355	0.345	0.290	0.289	1 849
	Length.....	24.3	24.4			31.4	31.5			27.9
No. 28a	Fresh weight.....	0.226	0.228	0.145	0.147	0.487	0.454	0.400	0.380	2 467
	Dried weight.....	0.162	0.177	0.123	0.125	0.331	0.313	0.288	0.286	1 805
	Length.....	24.8	24.7			31.8	31.8			28.3
Control (2)	Fresh weight.....	0.205	0.196	0.128	0.118	0.440	0.429	0.331	0.319	2 166
	Dried weight.....	0.150	0.151	0.110	0.110	0.318	0.317	0.254	0.254	1 664
	Length.....	23.4	23.4			29.5	29.6			26.5
No. 30a	Fresh weight.....	0.218	0.209	0.143	0.132	0.461	0.449	0.365	0.355	2 332
	Dried weight.....	0.157	0.160	0.119	0.119	0.323	0.316	0.275	0.277	1 746
	Length.....	23.7	23.8			30.5	30.4			27.1
Control (2)	Fresh weight.....	0.189	0.179	0.121	0.110	0.407	0.398	0.304	0.289	1 997
	Dried weight.....	0.142	0.142	0.106	0.104	0.296	0.298	0.236	0.231	1 555
	Length.....	23.7	23.6			30.1	30.1			26.9
No. 31a	Fresh weight.....	0.221	0.212	0.145	0.136	0.461	0.457	0.357	0.360	2 349
	Dried weight.....	0.163	0.165	0.125	0.125	0.328	0.335	0.271	0.277	1 789
	Length.....	25.0	25.0			31.7	31.7			23.4
Control (6)	Fresh weight.....	0.204	0.198	0.130	0.122	0.448	0.435	0.339	0.330	2 206
	Dried weight.....	0.151	0.152	0.113	0.112	0.323	0.320	0.260	0.258	1 689
	Length.....	23.8	23.8			30.3	30.4			27.1
Average	Fresh weight.....	0.222	0.216	0.144	0.138	0.470	0.454	0.374	0.365	2 383
	Dried weight.....	0.161	0.167	0.122	0.123	0.327	0.321	0.278	0.280	1 779
	Length.....	24.5	24.5			31.3	31.3			27.9

TABLE 12

Giving the weight (gms.) and length (mm.) data for the semi-castrated albino rats compared with those for the controls. After semi-castration at 17-27 days

GROUP		NO. OF CASES		TAIL LENGTH		BODY WEIGHT		TESTES	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYS-TEM		PER CENT WATER		NO. OF DAYS AFTER OPER.	AGE DAYS
													Brain	Sp. Cd.	Brain	Sp. Cd.		
I	Control	4217	183	281.2	2.424	0.0481	0.0355	0.1864	0.0100	1.886	0.62178	57971.482	168					
	Operated	5203	163	209.1	1.329 ¹	0.0535	0.0323	0.1310	0.0083	1.810	0.57578	45871.999	150					
	Per cent: Oper.-cont	0	-1.2	-6.2	23.8	41.0	5.5	-28.7	0.52	-0.88	1.3	-0.12	0.52	168				
II	Control	5210	175	225.9	2.360	0.0365	0.0348	0.1540	0.0083	1.868	0.59678	27171.456	173					
	Operated	6202	171	202.8	1.281 ¹	0.0356	0.0320	0.1290	0.0081	1.842	0.57678	33771.798	149					
	Per cent: Oper.-cont	0	2.2	2.9	17.2	8.6	0.52	-13.3	8.0	0.50	2.0	0.07	0.34	173				
III	Control	4224	187	293.2	2.381	0.0617	0.0392	0.1802	0.0099	1.919	0.64978	36771.413	180					
	Operated	4210	180	237.3	1.198 ¹	0.0379	0.0350	0.1286	0.0087	1.831	0.60578	34771.601	153					
	Per cent: Oper.-cont	0	3.2	2.4	12.0	-37.3	4.1	-30.0	6.6	-1.5	1.7	-0.02	0.19	180				
IV	Control	5223	192	270.1	2.445	0.0746	0.0336	0.1648	0.0121	1.888	0.62178	62971.213	180					
	Operated	5218	191	277.0	1.326 ¹	0.0841	0.0334	0.1727	0.0119	1.864	0.60978	69771.433	158					
	Per cent: Oper.-cont	0	2.2	7.1	12.1	35.4	4.1	4.4	5.7	-0.15	1.1	0.07	0.22	180				
V	Control	4213	178	246.1	2.033	0.1429	0.0350	0.1826	0.0081	1.875	0.59678	52371.557	197					
	Operated	4210	182	241.3	1.061 ¹	0.1495	0.0335	0.1613	0.0102	1.859	0.61378	56572.056	180					
	Per cent: Oper.-cont	0	3.3	3.2	6.1	34.4	-0.87	-12.8	29.8	-0.17	5.1	0.04	0.50	197				
I-V	Control	22217	183	263.3	2.329	0.0728	0.0356	0.1736	0.0097	1.887	0.61778	47471.424	180					
	Operated	24209	177	233.5	1.239 ¹	0.0721	0.0332	0.1445	0.0094	1.841	0.59678	48171.777	158					
	Per cent: Oper.-cont	0	1.9	1.9	14.2	16.4	2.7	-16.1	10.1	-0.45	2.2	0.01	0.35	180				

¹ Weight of surviving testis.

TABLE 13
Giving the weight (gms.) and length (mm.) data for the semi-castrated albino rats compared with those for the controls. After semi-castration at 81 days.

GROUP	NO. CASES	BODY LENGTH		TAIL LENGTH		BODY WEIGHT		TESTES		THYROID		SUPRARENALS		THYMUS		HYPOPHYSIS		CENTRAL NERVOUS SYSTEM		PERCENT WATER		NO. DAYS AFTER OPER.		AGE DAYS	
		BODY LENGTH		TAIL LENGTH		BODY WEIGHT		TESTES		THYROID		SUPRARENALS		THYMUS		HYPOPHYSIS		CENTRAL NERVOUS SYSTEM		PERCENT WATER		NO. DAYS AFTER OPER.		AGE DAYS	
VI	Control	6	205	180	180	210.3	2.161	2.161	0.0499	0.0345	0.0997	0.0082	1.757	0.571	78.241	71.077	198								
	Operated	5	209	181	237.3	1.298 ¹	1.298 ¹	0.0422	0.0364	0.1159	0.0086	1.821	0.601	78.342	71.254	117	198								
	Per cent: Oper.-cont...	0	-1.8	5.4	15.1			-29.6	0.87	9.8	-0.25	2.5	2.4	0.10	0.18										

¹ Weight of surviving testis.

TABLE 14

Percentage weight of the parts of the encephalon in albino rats after semi-castration at 17-27 days.

	BODY LENGTH	BRAIN	CEREBRUM	STEM	CEREBELLUM	OLF. BULBS	NO.	AGE
Control.....	214	1.870	1.183	0.349	0.264	0.075	4	149
Per cent.....		100.0	63.3	18.6	14.1	4.0		
A. Operated.....	197	1.783	1.131	0.348	0.252	0.051	5	149
Per cent.....		100.0	63.5	19.5	14.2	2.8		
Control.....	218	1.881	1.171	0.366	0.270	0.074	4	161
Per cent.....		100.0	62.2	19.4	14.4	4.0		
B. Operated.....	207	1.855	1.172	0.362	0.265	0.057	4	161
Per cent.....		100.0	63.2	19.5	14.3	3.1		
Control.....	220	1.929	1.187	0.377	0.288	0.076	4	165
Per cent.....		100.0	61.6	19.5	14.9	4.0		
C. Operated.....	215	1.877	1.165	0.371	0.282	0.059	6	165
Per cent.....		100.0	62.1	19.9	15.0	3.1		
Control.....	217	1.887	1.203	0.366	0.269	0.050	5	195
Per cent.....		100.0	63.7	19.4	14.1	2.6		
D. Operated.....	206	1.802	1.136	0.363	0.262	0.040	5	195
Per cent.....		100.0	63.1	20.2	14.5	2.2		
Control.....	200	1.660	1.065	0.320	0.245	0.029	2	158
Per cent.....		100.0	64.2	19.3	14.8	1.8		
E. Operated.....	206	1.774	1.123	0.344	0.268	0.039	2	158
Per cent.....		100.0	63.3	19.4	15.1	2.1		
Control.....	207	1.806	1.112	0.369	0.275	0.050	2	202
Per cent.....		100.0	61.6	20.5	15.2	2.7		
F. Operated.....	209	1.847	1.138	0.373	0.281	0.054	2	202
Per cent.....		100.0	61.6	20.2	15.2	3.0		
Control.....	213	1.839	1.154	0.358	0.269	0.059	21	172
Per cent.....			62.8	19.5	14.6	3.2		
A.-F. Operated.....	207	1.823	1.144	0.360	0.268	0.050	24	172
Per cent.....			62.8	19.8	14.7	2.7		

TABLE 15

Giving the weight (gms.) and length (mm.) data for the operated rats compared with those for the controls. After ligation of spermatic cord at 24-26 days.

GROUP		NO. CASES	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	TESTES	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYSTEM		PERCENT WATER		NO. DAYS AFTER OPER.	AGE DAYS
											Brain	Sp. Cd.	Brain	Sp. Cd.		
I	Control.....	3	206	176	224.4	2.438	0.0464	0.0378	0.2508	0.0073	1.839	0.541				124
	Operated.....	3	201	170	207.2	1.314	0.0364	0.0336	0.1970	0.0073	1.842	0.538			98	124
	Per cent: Oper.-cont....	0	-0.57	0.59	-31.6		-21.9	-6.2	-20.2	6.2	1.3	2.6				
II	Control.....	3	193	163	197.0	2.145	0.0196	0.0314	0.1509	0.0070	1.858	0.537				118
	Operated.....	3	191	166	188.4	0.289	0.0240	0.0401	0.2649	0.0089	1.848	0.531			94	118
	Per cent: Oper.-cont....	0	3.1	3.1	10.5	-93.2	17.4	30.6	41.8	32.5	-0.05	0.38				

TABLE 16

Giving the weight (gms.) and length (mm.) data for the semi-spayed albino rats compared with those for the controls. After semi-spaying at 38-51 days.

GROUP	NO. CASES	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	OVARIES	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYSTEM		PER CENT WATER		NO. DAYS AFTER OPER.	AGE DAYS
										Brain	Sp. Cd.	Brain	Sp. Cd.		
V	Control.....	4	195	167	177.2	0.0533	0.0278	0.0437	0.1510	0.0094	1.745	0.53678	38071.263	184	
	Operated.....	4	198	180	177.4	0.0613 ¹	0.0289	0.0478	0.1207	0.0098	1.716	0.53978	55771.997	133	184
	Per cent: Oper.-cont....		0	2.9	-4.6	140.5	-0.18	3.9	-17.0	-0.19	-2.3	-1.4	0.18	0.73	
VI	Control.....	2	199	181	179.8	0.0512	0.0526	0.0479	0.1664	0.0142	1.796	0.55378	27170.909	200	
	Operated.....	3	199	177	207.3	0.0559 ¹	0.0748	0.0543	0.2070	0.0150	1.827	0.56678	52871.489	162	200
	Per cent: Oper.-cont....		0	-2.3	13.4	123.2	67.3	11.8	24.9	5.6	1.7	2.2	0.26	0.58	
V-VI	Control.....	6	197	174	178.5	0.0523	0.0402	0.0458	0.1587	0.0118	1.771	0.54578	32671.086	192	
	Operated.....	7	199	179	192.4	0.0586 ¹	0.0519	0.0511	0.1639	0.0124	1.772	0.55378	54371.743	148	192
	Per cent: Oper.-cont....		0	0.36	4.4	131.8	33.6	7.8	4.0	2.7	-0.29	0.40	0.22	0.66	

¹ Weight of surviving ovary.

TABLE 17

The weights (gms.) and lengths (mm.) of the bones together with the percentage of water in them in the semi-sprayed albino rats.

	BODY (1) WEIGHT (2) LENGTH	HUMERUS		RADIUS AND ULNA		FEMUR		TIBIA AND FIBULA		ALL BONES	AGE
		Right	Left	Right	Left	Right	Left	Right	Left		
Control (1) No. 45d	{ Fresh weight.....	0.203	0.204	0.125	0.124	0.455	0.445	0.349	0.346	2.251	162
	{ Dried weight.....	0.141	0.142	0.103	0.104	0.290	0.291	0.254	0.251	1.576	
	{ Length.....	24.9	24.8			32.0	32.2			28.5	
Operated (2) No. 45d	{ Fresh weight.....	0.207	0.204	0.130	0.130	0.457	0.452	0.345	0.349	2.274	162
	{ Dried weight.....	0.139	0.138	0.111	.110	0.279	0.278	0.241	0.240	1.536	
	{ Length.....	25.0	24.9			32.1	32.1			28.6	
Control (2) No. 16d	{ Fresh weight.....	0.245	0.246	0.158	0.156	0.537	0.529	0.410	0.403	2.684	200
	{ Dried weight.....	0.180	0.182	0.137	0.137	0.385	0.382	0.313	0.312	2.028	
	{ Length.....	199									
Operated (3) No. 16d	{ Fresh weight.....	0.250	0.246	0.159	0.156	0.540	0.537	0.411	0.408	2.707	181
	{ Dried weight.....	0.189	0.189	0.140	0.139	0.396	0.401	0.323	0.325	2.102	
	{ Length.....	199									
Control (3) Average	{ Fresh weight.....	0.224	0.225	0.142	0.140	0.496	0.487	0.380	0.375	2.468	181
	{ Dried weight.....	0.160	0.162	0.120	0.121	0.338	0.337	0.284	0.282	1.802	
	{ Length.....	24.2	24.2			30.6	30.6			27.4	
Operated (5) Average	{ Fresh weight.....	0.229	0.225	0.145	0.143	0.499	0.495	0.378	0.379	2.491	181
	{ Dried weight.....	0.164	0.164	0.126	0.125	0.338	0.340	0.282	0.283	1.819	
	{ Length.....	24.2	24.3			31.5	31.5			27.9	

TABLE 18
Giving the weight (gms.) and length (mm.) data for the operated albino rats compared with those for the controls. After isolation of one ovary and removal of the other at 27 days.

GROUP		NO. CASES		BODY LENGTH	TAIL LENGTH	BODY WEIGHT	OVARIES	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYSTEM		PER CENT WATER	NO. DAYS AFTER OPER.	AGE DAYS
												Brain	Sp. Cd.	Brain	Sp. Cd.	
I	Control	8	198	168	186.0	0.0552	0.0418	0.0561	0.1330	0.0128	0.0152					202
	Operated	10	198	170	193.2	0.0378 ¹	0.0482	0.0509	0.1558	0.0152	0.0152					175
	Per cent: Oper.-cont.	0	1.2	3.6	42.2		19.7	7.1	14.1	17.3						202
II	Control	7	195	171	173.5	0.0542	0.0483	0.0524	0.1895	0.0120	0.0107					209
	Operated	7	192	169	165.4	0.0415 ¹	0.0480	0.0495	0.1955	0.0107	0.0107					182
	Per cent: Oper.-cont.	0	0.60	0.47	60.4		5.2	0.94	3.9	-5.3						209
I-II	Control	15	197	170	179.8	0.0547	0.0451	0.0543	0.1613	0.0124	0.0130					206
	Operated	17	195	170	179.3	0.0397 ¹	0.0481	0.0547	0.1757	0.0130	0.0130					179
	Per cent: Oper.-cont.	0	0.88	2.0	51.3		12.4	4.0	9.0	6.0						206

¹ Weight of surviving ovary.

TABLE 19
Giving the weight (gms.) and length (mm.) data for the operated albino rats compared with those for the controls. After double isolation of ovaries at 31 days.

GROUP		NO. CASES		BODY LENGTH	TAIL LENGTH	BODY WEIGHT	OVARIES	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	CENTRAL NERVOUS SYSTEM		PER CENT WATER	NO. DAYS AFTER OPER.	AGE DAYS
												Brain	Sp. Cd.	Brain	Sp. Cd.	
I	Control	8	191	166	165.2	0.0483	0.0569	0.0447	0.1627	0.0122	0.0122	1.661	0.508	78.453	71.447	198
	Operated	6	194	168	175.4	0.0555	0.0526	0.0443	0.1755	0.0123	0.0123	1.707	0.541	78.525	71.338	167
	Per cent: Oper.-cont.	0	-0.58	0.66	14.0	-21.5	-5.1		7.7	-3.9	-3.9	-1.9	3.7	0.07	-0.11	198

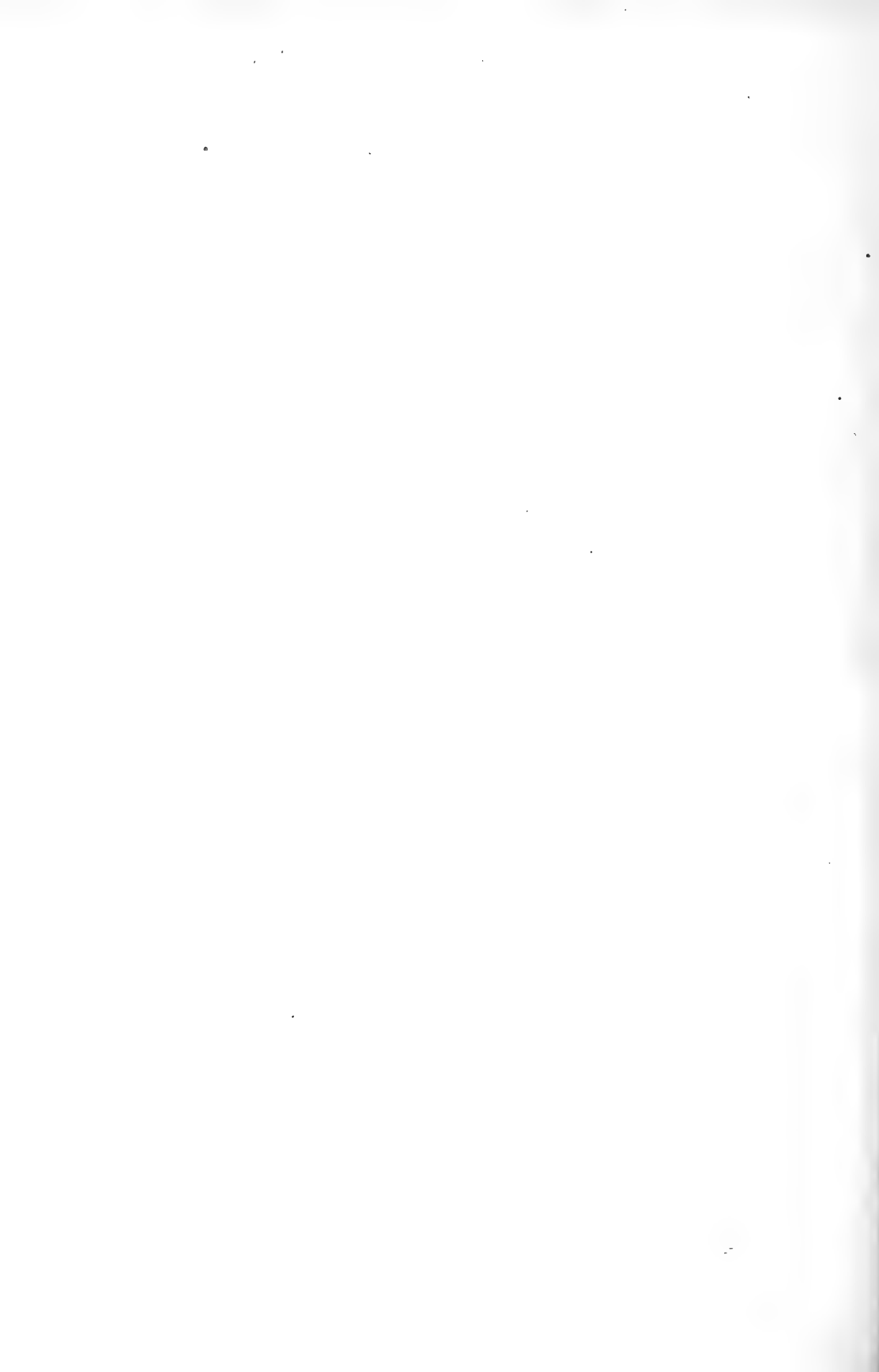
TABLE 20

Giving the percentage deviations of the various characters in the operated albino rats from the values found in the controls.

	NO. CASES	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	SEX GLANDS	THYROID	SUPRARENALS	THYMUS	HYPOPHYSIS	WEIGHT OF		PER CENT WATER		NO. DAYS AFTER OPER.	AGE DAYS
										Brain	Sp. Cd.	Brain	Sp. Cd.		
Castration.....	27 C.	0	4.7	6.0		-21.5	17.6	61.2	56.7	2.6	3.3	0.01	0.20	138	157
16-22 days.....															
Per cent: Oper.-Cont.....	26 O.														
Castration.....	6 C.	0	3.2	3.0		3.8	6.8	74.9	42.6	0.51	0.32	-0.17	0.29	111	197
79-93 days.....															
Per cent: Oper.-Cont.....	5 O.														
Castration.....	7 C.	0	1.8	5.4		-6.7	8.5	53.9	39.1	-0.74	0.08	-0.10	0.19	55	273
208-232 days.....															
Per cent: Oper.-Cont.....	8 O.														
Spaying.....	30 C.	0	1.9	7.3		3.3	-18.5	58.3	8.9	1.3	2.5	0.00	-0.30	133	158
19-30 days.....															
Per cent: Oper.-Cont.....	28 O.														
Spaying.....	9 C.	0	-1.5	6.4		-50.6	-25.0	39.6	7.8	0	1.5	-0.08	0.38	100	208
97-119 days.....															
Per cent: Oper.-Cont.....	8 O.														
Spaying.....	9 C.	0	1.2	3.5		13.9	-5.3	27.5	10.4	-1.5	0.16	0.01	0.13	41	221
172-195 days.....															
Per cent: Oper.-Cont.....	7 O.														

TABLE 20—Continued

Semi-castration	22C.	0	1.9	1.9	14.2	16.4	2.7	-16.1	10.1	-0.45	2.2	0.01	0.35	158	180
17-27 days.....															
Per cent: Oper.-Cont	24O.														
Semi-castration	6C.	0	-1.8	5.4	15.1	-29.6	0.87	9.8	-0.25	2.5	2.4	0.10	0.18	117	198
81 days.....															
Per cent: Oper.-Cont	5O.														
Semi-spaying.....	6C.	0	0.36	4.4	131.8	33.6	7.8	4.0	2.7	-0.29	0.40	0.22	0.66	148	192
38-51 days.....															
Per cent: Oper.-Cont	7O.														
Single isolation.....	15C.	0	0.88	2.0	51.3	12.4	4.0	9.0	6.0					179	206
27 days.....															
Per cent: Oper.-Cont	17O.														
Double isolation.....	8C.	0	-0.58	0.66	14.0	-21.5	-5.1	7.7	-3.9	-1.9	3.7	0.07	-0.11	167	198
31 days.....															
Per cent: Oper.-Cont	6O.														



EXPERIMENTAL STUDIES OF HYBRIDIZATION AMONG DUCKS AND PHEASANTS

JOHN C. PHILLIPS

NINE PLATES

INTRODUCTION

The following experiments were started in 1909 and 1910 with the idea of studying plumage characters in wild species of birds. All the following crosses may be regarded as between wild species, except the Black East India experiment, the 'freak' mallard experiment, and the albino pheasant experiment.

In the ducks the mallard has always been used as one of the parents. These mallards were all from the same strain as that used in the size crosses (24). They are a pure strain of English mallards, such as are used in English game parks. During the years in which this strain has been bred here, about 500 of these birds have been reared. No departure from type has been noted except two freak ducklings in 1909 (see 'freak' mallard experiment), and one in 1913.

Reciprocal duck crosses were attempted with the mallard as male parent and the black-duck, pintail, and Australian as the female parent, but in the small mating pens it was found impossible to induce the females of these latter species to lay.

It is scarcely necessary to describe the mallard duck. The mallard characters dealt with in the following pages are, green head, white neck ring, chestnut breast, curly sex feathers in the tail, vermiculated abdomen and flanks, and white anterior and posterior bars to the purple speculum of the wing. Each experiment is described separately in the following pages.

PINTAIL MALLARD EXPERIMENT

A cross between two sexually dimorphic species

In 1910 two pure wild male pintail ducks (*Dafila acuta*), Nos. 108 and 109, were placed in a mating pen with two female mallards, Nos. 141 and 142, from the original mallard stock described above (cross R 1910). From this mating a large number of eggs were gathered, but only seven individuals hatched out, of which six were reared to maturity, male 105, and females 102, 103, 104, 91, and 72. The appearance of hybrids produced by this cross has often been described, but will be given in detail later on. Both males and females are distinctly intermediate in color, shape, and general carriage, but the females of the parent species do not differ in any striking peculiarities, so that the female hybrids may be disregarded in this account.

During the years 1911, 1912 and 1913, a number of F_2 birds were reared from the six F_1 ducks of 1910. Only one mating could be made each year on account of having only one F_1 male, so that the numbers reared are not as large as could be wished. The F_1 ducks do not lay freely, at least not in the space which could be accorded to them. In all there are only sixteen F_2 males.

In 1913 a large number of pure wild male pintail ducks were placed in a large pen together with a number of female mallards of exactly the same stock used in the first cross (1910). From this mating about a hundred birds were reared to maturity and forty-six F_1 males were carefully studied (cross N 1913).

The skins of eleven F_2 hybrids and seven F_1 hybrids were preserved and notes made on all the other specimens. It may be remarked in passing that there is a very striking resemblance between F_1 and F_2 birds, and, except for one or two characters, there does not seem to be any more tendency to variation among F_2 than among F_1 hybrids. Plates 1 and 2 show males of the parent races, and the extreme variations of both generations. As there was a greater choice of numbers among the F_1 birds (forty-six as against sixteen), a tendency to greater variation among the F_2 's than is here shown cannot be entirely ruled out, but the F_1

material may be considered as ample. In general it must be acknowledged that the first generation hybrids seem to have rather less of the mallard chestnut breast area than the second generation hybrids, but the difference is slight.

The pintail duck represents a genus of true ducks distinguished by their characteristic shape and elongated tails. The head is brown and the breast white, the white pointing far up the sides of the neck. The bill is lead blue, black on the nail and very different from the color of the mallard's bill. The mantle and flanks are finely vermiculated with gray and black. There is a black area on the scapular feathers, and the middle tail feathers are very long and thin. The speculum is green, framed in front by a brown bar and behind by a white one.

The typical F_1 hybrid may now be described and the variations from this type afterwards indicated (pl. 1, fig. 8; pl. 2, fig. 4).

F_1 male No. 966 (cross N 1913) and type of F_1 generation. The bill is practically pure pintail in color, being a dull lead blue, black on the nail. In size and shape, however, the bill is probably exactly intermediate, as pointed out by Bigelow (1). The color of the head is a very dark purplish brown with a metallic gloss of green, which is much brighter on the post-ocular and hind-neck region. The crown of the head is more brownish and directly under the eyes is a small semilunar spot of white. Therefore the head color shows both the brilliant green of the mallard and the plain brown of the pintail.

The upper breast has the chestnut area of the mallard, but reduced both in extent and in depth of color (pl. 9, fig. 8), and not sharply marked off posteriorly, where it fades out into the pure white of the abdomen. This condition is exactly half way between the white upper breast of the pintail and the chestnut breast of the mallard (pl. 9, fig. 3). The chestnut area of the hybrid is sharply separated from the dark colored head by a white collar, which is wider than that of the mallard and tends to point up on the sides of the neck as in the pintail. The silver gray undulated appearance of the flanks and the plain gray color of the lower abdomen are absolutely intermediate. The under tail coverts are black as in both parent species. The

mantle is gray with very fine black vermiculations, halfway between the coarser vermiculations of the pintail and the plain dark umber brown of the mallard. The rump is very dark, almost black, as in the mallard, but preserves a few of the lighter gray feathers of the pintail. The middle tail feathers have the elongation of the pintail and the upcurling of the mallard, but both characters in a modified form.

The speculum of the wing is very interesting, being a brilliant clear metallic green, nearest to the Veridian green of Ridgeway ('12). This is very unlike the purple blue of the mallard or the bronzy green to coppery color of the pintail, but more than likely it is directly due to an intermediate condition. The rest of the speculum preserves in a slightly modified form the anterior brown wing bar of the pintail, while its white posterior wing bar is not so wide as in the pintail, but wider than in the mallard. The legs and feet are dull orange yellow, not the brilliant orange of the mallard or the grayish black of the pintail.

I do not agree with Bigelow in finding any pure parental characters in the hybrid, with the possible exception of the color of the bill, and this is as often as not modified by a spot of dull yellow at the base of the culmen.

The rufous markings of the upper tail coverts, described in Bigelow's wild specimen, are not always present, and are scarcely important enough when present to warrant calling them a new character. The description of this hybrid could be made more complete, but the most important points have been enumerated.

The F_1 generation males show a measurable amount of variation, this variation being greatest in the chestnut breast area (pl. 1, figs. 7, 8, 9; pl. 2, figs. 3, 4, 5), in the form of the neckring, and in the color of the rump. The first two of these characters are ones that tend to vary most in the pure wild mallard and in domestic varieties. Also there is a slight range in the vermiculated appearance of the mantle. The colors of the head, the flanks, and the lower abdomen are very uniform. When the mallard chestnut breast area is much reduced, as in F_1 male 849 (pl. 1, fig. 9), there is always a widening of the white collar and a tendency for it to point forward along the sides of the neck,

as in the pintail. There is always correlation between these characters, but this cannot be said of the mantle pattern and the rump color. The bill color runs from lead blue to yellowish blue, and the legs and feet from dull orange yellow to dull orange. The plate shows the total range of variation in the breast and neck areas, as seen in the two most divergent of the males. In ♂ 849 (pl. 1, fig. 9), the chestnut area is very light colored, the feathers tipped with white, and the region fading into the pure white of the pintail breast. The white collar is 1.5 cm. wide, ventrally, and over 3 cm. wide dorsally. In the other extreme, F₁ ♂ 853 or ♂ 967, the chestnut area is nearly as great in extent as in the mallard, though less intense in color, while the collar is reduced to about the same size as that of the mallard parent (pl. 1, fig. 7). Between these types the other forty-five specimens show every possible shade and degree.

F₂ generation: On the whole this appears to lean towards the pintail parent rather more than the F₁ lot. The extreme bird is F₁ ♂ 525 (pl. 1, fig. 11; pl. 2, fig. 7). In this specimen the chestnut area is reduced to a slight stain on the sides of the upper breast, while the lower parts, except the abdomen, are practically pure white. The white nape stripes of the pintail are very highly developed, as is the black nuchal area between them, while the whole head is much browner in color than that of any other specimen in either generation (this does not show in the plate). The mantle is very clearly vermiculated, and the rump light in color. The bill was lead blue in life, but with an orange spot at the base, while the legs and feet were pale dirty straw color. This specimen is certainly a distinct, though slight departure from the most extreme variation in the first hybrid generation. This specimen was almost duplicated by ♂ 1217 (1913).

The extreme F₂ variation in the mallard direction is ♂ 530, 1912 (pl. 1, fig. 10), in which the white collar is much reduced, and the head rather a brilliant green. It can be said with certainty that this bird shows a slightly greener (more mallard) head than any other specimen in either generation. Its bill was bluish lead color, and the legs and feet a dull orange. This bird was closely approached by ♂ 1218 (1913).

It has been noticed that the extreme F_2 variates are slightly farther removed from each other than are the variates of the first generation, although the smaller numbers of the F_2 's are against this result. The differences are very slight and not clear cut. Between the two F_2 extremes there fall the fourteen other specimens, the enumeration of which would take too much space. The bills of this series run from the pure lead-color type seen in many specimens, to a dull greenish yellow in ♂ 675 (1912), showing on the whole a tendency to a little more range of color than the previous generation. The same thing is seen in the feet and legs, which run from a dirty straw color to a dull orange.

The sex feathers in this F_2 generation are much longer and better developed than in the F_1 's, but this, I believe, is entirely due to the fact that the F_2 's were allowed to grow older before being killed. There is a marked tendency for sex feathers to develop late in all mallard hybrids. It is certain that these F_2 individuals were far less vigorous than the F_1 's and their plumage developed later. In the best developed F_2 specimen the 'pin' of the tail is as long as in the pintail parent, but it is curved up to about half a circle, not tightly curled as in the mallard.

F_1 specimens of the 1913 hatch are now alive (February, 1914), and show tail feathers much longer than individuals killed for specimens in November.

Back-cross, wild pintail ♂ × pintail-mallard F_1 ♀

This cross was made in 1913, a wild pintail male being mated with two of the original F_1 females (cross F, 1913). As a result three males and six females were reared to maturity (pl. 1, figs. 5 and 6). Two of the males and two females are still alive. On casual inspection the plumage of the males appears pure pintail, although the shape of the bird is distinctly mallard-like, the neck being short and the body chunky. This shape applies to both sexes. More careful inspection shows the head to be darker than the pintail, especially in throat and chin, with a slight iridescence lacking in the pintail. The black scapular patch of the pintail is almost entirely lacking, while the tail feathers

are not plain, but irregularly barred. This last character is hard to account for, as it does not occur in either parent or in the first generation hybrids. Besides this, the upper tail coverts have considerably more black externally, extending even into the inner web in some cases. On the upper chest is a very faint blush of pink, barely noticeable, but without question the remains of the mallard chest area (pl. 9, fig. 11). The females are not easily distinguished from pintails, but they are browner.

Measurements bear out the observation that the $\frac{3}{4}$ pintails are chunkier than either pure pintails or F_1 hybrids. The following measurement, found to be a very uniform one, is the distance from the anterior point of the breast bone to the tip of the bill (Jour. Exp. Zool., vol. 16, p. 131) neck length:

Pintail	♂	32.7	♀	29.0
Mallard	♂	30.5	♀	26.8
F_1	♂	32.0	♀	27.4
$\frac{3}{4}$ pintail	♂	30.2	♀	26.0

Summary. There is thought to be a slight though definite tendency to more variation in F_2 hybrids than in F_1 hybrids in this cross. The first hybrids are shown to be as exact an intermediate in every detail as could possibly be imagined. They are shown to vary to a measurable extent, mostly in those characters that tend to vary in the mallard parent, the chestnut breast and white neck ring.

The F_2 hybrid material is not sufficient to establish beyond question the occurrence or non-occurrence of distinct Mendelizing characters, but indicates that Mendelizing units of a simple sort do not occur. It is expected that the number of F_2 hybrids will be largely added to. Small as it is, it demonstrates the point stated above. It is doubtful whether any reasonable number of specimens of this generation will give any new types, but this can only be proved by further work.

The tendency to a more pintail appearance of the F_2 's cannot be accounted for, as the original F_1 ♂, still alive, is a good F_1 type.¹

¹ Cross J 1914, ♂ (♂ pintail × ♀ F_1 pintail × mallard) × ♀ mallard gave 16 ♂♂ which form a very uniform series, but show slightly more pronounced chestnut breasts than F_1 ♂♂. They are $\frac{3}{4}$ pintail and $\frac{1}{4}$ mallard.

In the back cross with the pintail parent the female F_2 hybrids have transmitted from their mallard mothers distinct mallard characters, involving both shape (cervical vertebrae length), color, and color pattern.

Black duck (Anas tristis) and mallard crosses

This cross involves the union of two species, only one of which has sex dimorphism; i.e., the mallard. It is analagous to the Australian-mallard crosses to be described next.

The black duck (pl. 3, fig. 3) is brownish black all over, the feathers margined with fulvous or gray. Both sexes are alike. The speculum of the wing is the same color as that of the mallard but it lacks the white bars, although a posterior one is sometimes developed to a slight extent. The top of the head is nearly black, and there is a dark post-ocular streak. The rest of the head and neck, down to that region which in the mallard is marked by a white collar, is grayish-fulvous streaked with dusky. This is perhaps the plainest colored species of the true ducks.

The cross was started in 1909, when two wild male black ducks were mated with two female call ducks. These call ducks came from a strain which had been bred by me for several generations, and showed no departure from the general mallard type, although somewhat more variation exists than among the English mallard stock used in all the other duck crosses. For purposes of convenience the call ducks may be regarded as pure mallard. They are in point of fact a rather coarsened mallard, with a more upright carriage, a higher voice, and a tendency to throw darker individuals occasionally.

The result of the 1909 cross was an F_1 generation composed of three males, Nos. 19, 22, 23; and four females, Nos. 24, 25, 26, and 27. These F_1 birds (pl. 3, fig. 4) were kept for several years, so that all delayed appearance of mallard characters may be ruled out. They may be described as dark intermediate types, showing plainly all the mallard characters, but obscured by the plain, dark brownish color of the black duck parent. The only mallard character which is strongly developed is the curly sex

feathers in the tail, and these are not quite so long as in the mallard. To describe this hybrid more in detail: the post-ocular and hind-neck region is brilliant mallard green, the occiput is black, with a slight green iridescence, while the sides of the face, neck, throat, and chin are buff, spotted with black, like the black duck parent. The breast shows the mallard chestnut, but much obscured and less brilliant, while the feathers of this area have a broad, black, sub-terminal pattern (pl. 9, fig. 6). The lower edge of the chestnut breast area is poorly marked off from the abdomen, not by a sharp line as in the mallard, and the white underparts have a dark sooty appearance, with the fine vermiculated pattern of the mallard showing through to some extent. The under tail coverts are mottled and intermediate in type. The whole upper surface is very close to the black duck, but the speculum of the wing has the white anterior and posterior bars of the mallard. The rump has a greenish tinge, and the sex feathers are well developed (not so much so in the first year).

The female hybrid, as might be expected, is simply a dark mallard female with no distinctive characters, as there are no striking differences between the female parents (pl. 3, fig. 5).

It is of interest to note that this hybrid is very similar to the Australian-mallard hybrid, only in this latter cross all the mallard characters are less well developed (green head, sex feathers, chestnut area, etc.). This point will be referred to again.

In 1910 this entire lot of F_1 (mallard-black hybrids) was run together, and produced six males and nineteen females. These males were kept until one and one-half years old, so as to give them a chance to develop any latent characters. The nineteen females were allowed to grow to an age of two or three months, but as they showed nothing of interest (same as F_1 's) one was saved as a specimen and the rest discarded. In 1911 ten more F_2 males and four F_2 females were reared from the same source. This comprises all of the F_2 generation. The twenty-three females were all very much alike, and similar to F_1 females. The sixteen males must be briefly described. Out of the entire lot there may be said to be only one variate which is especially marked. This

is a bird (σ 174, 1911, pl. 3, fig. 8) about halfway between the F_1 type and the mallard parent. This bird was kept until a year and a half old, and was tested with pure mallard females (see below). It has a very green head with dark mottling on the cheeks and sides of the neck; while chin and throat are iridescent black. There is no neck ring, but the breast is rich chestnut, slightly spotted. The rest of the lower parts are like those of F_1 males but lighter and more vermiculated. The sex feathers are fully developed, and the flanks, sides of the breast and scapulars show mallard vermiculations. Male 37 (pl. 3, fig. 7) is another variate, with slightly more pronounced mallard characters than the F_1 type. On the other hand, several, such as σ 172, 1911, are darker than F_1 types. The anterior white wing bar in this last bird is absent, and there are no mallard vermiculations anywhere.

A good deal of variation is seen in the size of the F_2 sex feathers. In four cases they are absent, in five cases they are small, in two other cases they are recorded as 'medium,' and in five cases they are large. There is then a very considerable range of development of this character from pure black duck to nearly pure mallard. This F_2 generation shows a far greater range of variability than the same generation or the Australian-mallard cross. Part of this may be due to the strain of call duck introduced, but it is hardly probable that all of it is. F_2 σ 174 is without doubt a segregate. To confirm this opinion, this male was mated in 1912 with pure mallard stock (cross G, 1912). Four males and eight females were reared to maturity, and showed by their appearance that their father was very nearly a potential mallard. Three of the males were pure mallards in appearance, but one had the anterior wing bar reduced and a few spots on the breast. The females were all pure mallards, except that in a few cases the anterior wing bar was reduced or nearly absent. When the offspring of this cross are compared with three-quarter blood mallards (see below) it is seen that they approach much nearer to mallards than do the three-quarter bloods.

In 1912 there were made two other matings in this same experiment, back crosses, producing three-quarter blood mallards.

A male mallard was mated with two female F_1 birds (cross A, 1912), and a male F_1 bird was mated with two female mallards (cross C, 1912). From cross A ten males and eight females were reared, and from cross C, nine males and five females (pl. 3, figs. 10 and 11).

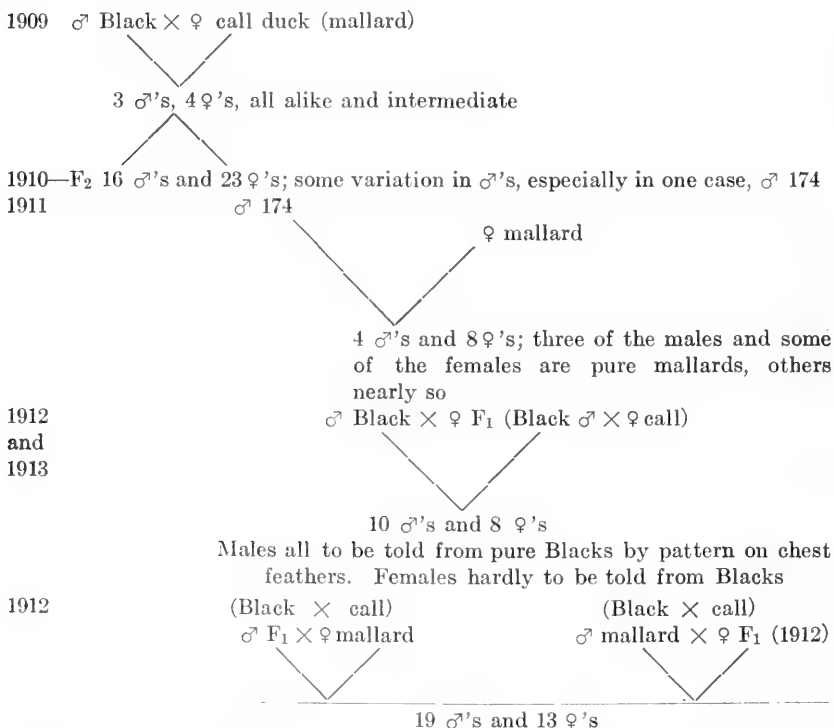
It is evident that in these crosses there was excellent opportunity for segregates to crop out, but out of the entire lot of nineteen males there are no pure mallards, and only one individual which can be called an F_1 type (σ^7 506, cross C). The results of both crosses are the same. They run from a modified F_1 type to mallard types with slightly spotted faces, reduced or absent neck rings, more or less spotted chestnut areas (pl. 9, fig. 5), darker upper parts, reduced white wing bars, and more or less coarseness and obscurity in the vermiculated areas. The sex feathers are always fairly well developed. A detailed account of this generation is not essential. Careful notes were made on each bird at maturity, and four specimens were saved.

In the same year, 1912, and also in 1913, a back cross was made between F_1 females and pure wild black duck males (pl. 3, fig. 9). Ducklings from this cross are not easy to rear, but there resulted in the two seasons ten males and eight females. These were kept until from six to eight months of age. It is difficult to say whether this lot could be picked out off-hand from a series of wild black ducks. Closer inspection shows that there is only one way that they can surely be told from pure black ducks, and this is by the pattern on the feathers of the upper chest (pl. 9, fig. 9). In all the males these feathers show, besides the terminal buff and sub-terminal black area, an inner distinct buff barring, which is further separated from the shaft by another blackish area. In the black duck parent this inner buff band is never present except in a very narrow or obscured condition (pl. 9, fig. 1). This barring is homologous to the 'eclipse' feathers on the chestnut area of the mallard's summer plumage (pl. 9, fig. 4) and is undoubtedly a mallard character (see Australian \times Mallard crosses). The females cannot be certainly told from pure black ducks. There is also great uniformity in this back cross.

The mallard eclipse breast pattern is seen in the males of those other species of black ducks which have slightly more differentiated sexes; i.e., *anas diazi* and *anas wyvilliana* and probably represents (23) a primitive sex dimorphism.

Besides this distinct mallard character in the three-quarter blood black duck hybrids reared from a female mallard, there is usually a slight tendency to a little green in the post ocular region, a slight development of sex feathers, and a darker rump, but these are not always present, and curiously enough they some-

TABLE 1

Scheme of matings: Mallard-Black duck experiment

Both crosses alike. They contain no pure mallards, but several ducks nearly pure. Only one individual can be classed as an F₁ type. The females are all to be told from pure mallards by reduced or absent white wing bars, either anterior or posterior, or both.

times occur in pure wild male black ducks (see discussion at end of Australian experiment). These very slight mallard characters would probably tend to increase a little with age, but this supposition has not been tested.

Summary. This cross results in hybrids very like the Australian-mallard crosses, but both in F_1 and F_2 and in the back cross with the black duck parent more mallard characters appear and apparently a greater amount of segregation occurs. This is probably due to the latent mallard characters present in the pure black duck. See Australian-mallard discussion, page 545. The back crosses, especially the three-quarter blood black duck series, are uniform, much more uniform than the F_2 generation. Out of sixteen ♂ F_2 's, only one segregate in the mallard direction occurred, and this bird when tested with pure mallard females produced apparently pure mallards in about half his offspring.

Back crosses never produce pure parental types except in the female sex.

AUSTRALIAN-MALLARD EXPERIMENT

Another cross between two species, only one of which is sex dimorphic

In 1909 a pair of Australian ducks, *Anas superciliosa*, was purchased (pl. 4, fig. 1). They were placed in a mating pen and produced seven ducklings, all of which were reared to maturity. Since that time the original pair has produced one or two broods each year, so that at least sixty of their offspring have passed through my hands, all of them proving absolutely true to type and showing no variations.

The Australian duck, a species ranging from Java through the Australia-New Zealand region to Polynesia, is a bird with rather local habits. For no very good reasons it has been placed in the genus *Polionetta*. It is very plain colored, the sexes being identical, and in a general way it closely resembles our American black duck, except for the pattern on the sides of its face, which at once differentiates it. This pattern consists of a very light buff colored super-ocular streak with a broad black ocular stripe under it. Below this is another buff stripe, separated from the

plain buff of the chin and throat by a narrow blackish line which runs from the gape to the ear coverts. The speculum is glossy green, not purple as in the mallard and black ducks, and framed both anteriorly and posteriorly by a broad black band. The legs and feet are dirty yellow, and the bill plumbeous color with a black nail. In size it is slightly smaller than the black duck and the mallard.

In 1910 two young ♂ Australians were mated with two ♀ mallards from original mallard stock (cross M, 1910), and produced four males and six females, ♂'s Nos. 88, 69, 77, 70; ♀'s Nos. 92, 83, 85, 86, 87, and 68.

The next year, 1911, F₁ ♂ No. 69 was mated with F₁ ♀'s Nos. 92 and 83 (cross C, 1911). As a result nineteen ducklings were hatched, but six died and only thirteen were reared to maturity. Of these only five were males. The females were examined and found to be all exactly similar to the F₁ females, except for a slight tendency to a difference in shade of the underparts. They need not be mentioned again. In the year 1912, ten more F₂ ♂'s were produced in the same way. In 1913 a pure Australian ♂ was mated with one of the original F₁ hybrid females (cross T, 1913), and nine birds of three-quarter Australian blood were produced. These will be described later. The skins of a number of specimens were preserved.

A reciprocal cross (♂ mallard × ♀ Australian) has thus far failed.

The first generation hybrids may now be briefly described (pl. 4, figs. 3 and 4). The female F₁ is remarkably like its Australian parent, and would quite easily be taken for that species by a careless observer. The bridle pattern on the face is, however, more obscured, the face being darker and more streaked, while the speculum is more purple, like the mallards. On the whole, though, there is a marked dominance of the darker Australian parent, like the black duck in the last experiment.

The four ♂ hybrids are all practically alike, except for age changes to be noted further on. They show very little of the mallard except a general lightness of tone, being lighter on the underparts than the female F₁'s. The face markings are the

same as in the female, the upper parts are similar, but the speculum is more mallard-like in appearance, having the broad anterior white bar of the mallard fully developed while the posterior white bar is reduced. This is all the more curious because its Australian parent has a distinct white posterior bar but no anterior one. There are, besides these marked characters, a very slight indication of the chestnut breast of the mallard and a distinct darkening of the rump and upper tail coverts. There is scarcely any tendency to mallard sex feathers, but the under tail coverts are sprinkled with buffy edgings to the feathers. This, it must be borne in mind, is the appearance of the hybrid at the age of one and a half years. At two and a half years, ♂ 77 (pl. 4, fig. 3) has developed somewhat his mallard tendencies. His breast has become more chestnut and his abdomen lighter, the chin and throat are a rich buff, more ruddy than at first, and the buffy under tail coverts are more developed. The sex feathers are distinctly developed, while at the base of the neck, underneath, is a small spot of white, indicating the position of the mallard neck ring. It is thus apparent that age tends to bring out the mallard sex characters. Age tends to make this hybrid more and more like the mallard-black duck hybrid, which from the close color relationship between the black duck and Australian duck it should certainly resemble. As a matter of fact, however, it never develops the marked green of the postocular region or the well-developed sex feathers which are always seen in the mallard \times black duck crosses, even at the first plumage. This point will be referred to later.

The first hybrid in this cross is then a bird intermediate in appearance but tending towards its darker parent, especially in the female. The secondary sex characters of the mallard become more prominent in the hybrid with age, at least up to the third autumnal moult; possibly longer. The female apparently does not change with age.

The F_2 generation, comprising fifteen males, need only be briefly considered. It is not possible to answer the question as to whether they show any tendency to a segregation of parental characters. If they do, it is only a slight tendency. The most

marked mallard variate is not nearly so mallard-like in appearance as the old F_1 bird referred to above (σ 77, 1909), but none of this generation were kept over one year. Age would undoubtedly tend to accentuate the mallard male characters as was shown for the first generation hybrids.

It may be remarked that F_2 σ 304, 1911, is rather Australian looking, and the speculum is distinctly greenish (pl. 4, fig. 7). It has, however, very distinct sex feathers and brown under tail coverts.

The anterior white specular bar tends to vary in this generation, being almost obsolete in σ 241, 1910, and fully up to a mallard standard in σ 68, 1910. This tendency to variation anterior to the speculum is seen in some pure wild species of ducks (23). It can be safely said that it would take a very large series of both generations, with the individuals kept for at least three years, to conclusively round out this experiment and measure the actual amount of segregation (if such a condition exists) in the second generation hybrids. As far as this experiment has gone there is nothing to show any greater variation in F_2 than in F_1 . Segregation, if it exists at all, is probably comparable to that found in the pintail-mallard crosses (a barely measurable amount).

The back cross produced in 1913 is of considerable interest. This consisted of a mating between a σ Australian and a female F_1 Australian \times mallard (cross T, 1913), as noted above. On first inspection they look pure Australian, and the females cannot with certainty be told from Australians, but the males all have the barred breast feather, seen in the three-quarter black ducks (pl. 9, fig. 10). This pattern was shown in the account of the black duck crosses, to be a mallard character, and is common also to certain other mallard-like ducks. There is also in these back cross birds a brownish edging and a brown barring of the under-tail coverts, seen more fully in F_1 's.

In comparing the males of this cross with the males produced in the black-duck \times mallard experiment a very considerable difference is found to be always present. The males of the three-quarter blood black ducks always show some mallard coloring (green post-ocular regions, iridescent rump or upper tail coverts,

more or less developed sex feathers, etc.). These characters appear in the young birds at the first adult plumage and very probably increase with age. We are thus led to the belief that the wild black duck actually contains mallard characters not present in the Australian duck, and that these are slightly accentuated by association in the zygote with characters from the mallard parent. The three-quarter blood pintail hybrid produced in the same way is not quite the same case, as both parent species are sexually dimorphic to a marked degree.

Certain variations of the black duck have received a great deal of attention from ornithologists, and space forbids a discussion of them here. It is sufficient to say that a second race of black ducks has been named on the evidence of several rather trivial characters; viz.: size, color of legs, color of pelium, streakiness of chin, etc. Various papers in the *Auk* by Brewster, Dwight, and Townsend have presented the pros and cons of this case. It has been held that the larger bird with the red legs migrates later and probably breeds in a more northern region. The validity of this race is still in dispute, and its name, *A. rubripes*, is now thrown into synonymy and but one species recognized. I pointed out in a note on the American black ducks (23, p. 300) that male black ducks kept in confinement for three years tended to develop quite marked mallard characters. Some of these characters are the very ones which are found in the three-quarter black duck hybrids, but not in the three-quarter Australian hybrids. In other words, there is present in the wild black duck, latent mallard characters, which sometime appear with age. These characters become immediately apparent when a quarter dose of mallard is combined with a three-quarter dose of black duck. The coral red legs of many adult wintering black ducks, which have caused so much discussion, may be partly an age character. We know, however, that coral legs do not always appear with age in the black duck (29, p. 176). The question of the two possible races of black ducks is brought up to show that the presence of mallard age characters in the black duck makes the systematic study of its two possible races still more complicated.

BLACK EAST INDIA DUCK CROSSED WITH MALLARD

In the winter of 1910 two pairs of the so-called East India ducks were purchased from G. D. Tilley. This race is apparently nothing but a simple melanistic derivative of the domestic mallard, and in no sense a wild species. It is a small cousin to the large black Cayuga duck, and resembles that bird in color. Both sexes are black all over, but the male has a fine metallic lustre which the female for the most part lacks. This breed is liable to show white feathers (30) especially with age, on the breast, neck ring region, and at the base of the lower mandible. In fact it is rare to get a bird that does not show some defect (white feathers) in later life, though the first plumage may be perfect. In poorer examples of the breed the mallard chest area shows through the black coloring. The race breeds perfectly true, as was amply demonstrated by my strain in 1911 and 1912; but, of course, there is variation in depth of color and metallic lustre. The eggs of this variety are a deep chocolate or sooty tint, but they grow lighter colored after the first few eggs are laid.

In order to see how this blackness behaved in crosses, a reciprocal cross was made with my original mallard stock in 1910. ♂ East India \times ♀ Mallard (cross J) produced ten offspring, six of which were saved for further work, ♂'s Nos. 72, 73, 129; ♀'s Nos. 71, 76, 131.

In the other cross (cross H, 1910) a male stock mallard was mated with the two ♀ East India ducks. Twelve birds were reared to maturity, and ♂♂ Nos. 121, 119, 81; and ♀♀'s 77, 78, and 80 were saved. As both sets of the F_1 hybrids were alike, and as both produced similar F_2 offspring, they may now, for the sake of simplicity, be treated together.

The first generation ducklings are black, except for a yellowish breast area, and sometimes an ocular yellow mark or a little yellow at the base of the bill. The adults are also black like the East India parent. Some of these hybrids are exactly as black as the black parent, but others show a slight brownish cast, especially among the females. One male (Cross J) has a large

white chest patch and very much lighter under parts. He was not used in producing the F_2 lot. The East Indian duck therefore shows almost complete domination over the mallard.

The F_2 generation was reared in 1911 and 1912, In the first of these years the reciprocal crosses were kept separate, as noted above, but as it evidently made no difference in which direction the cross was made (no sex linkage) this precaution was abandoned in 1912.

The entire F_2 generation considered together shows immediately after hatching two entirely different types with a questionable third type. There are sixty-one of the dominant East India type, twenty-eight mallard type, and four, jet black, so called 'freak type.' Forty-five ducklings of these three classes were reared to maturity and the rest were described in the down color and discarded for lack of space. All four of the 'freak' type were reared to maturity. The proportionate distribution of the types by years are, in the down, as follows:

	<i>Mallard</i>	<i>East India</i>	<i>'Freak' type</i>
1911.....	17	31	3
1912.....	11	30	1
Totals.....	28	61	4

We may now consider the dominant F_2 class. These show in the down a great variety of pattern, especially about the head. The post-ocular and occipital region is in some cases much mottled with yellowish, in other cases it has the same appearance as the F_1 or the pure East Indian duckling. Moreover, the upper breast and a patch at the base of lower mandible is apt to be more or less blotched with white, and the extent of this blotching was found to be a very rough indication of the blotchiness of the adult bird. The females proved more uniform in down.

At maturity the males of this dominant class were found to present a wide range of pattern, but the females were practically true to the F_1 type. This difference in the sexes is very marked. The males fall into a nearly continuous series from a type showing a distinct mallard pattern (though very dark) to a pure F_1 type (solid black). The males also exhibit all degrees of blotching at the neck-ring, chin, and on the breast, especially at that line

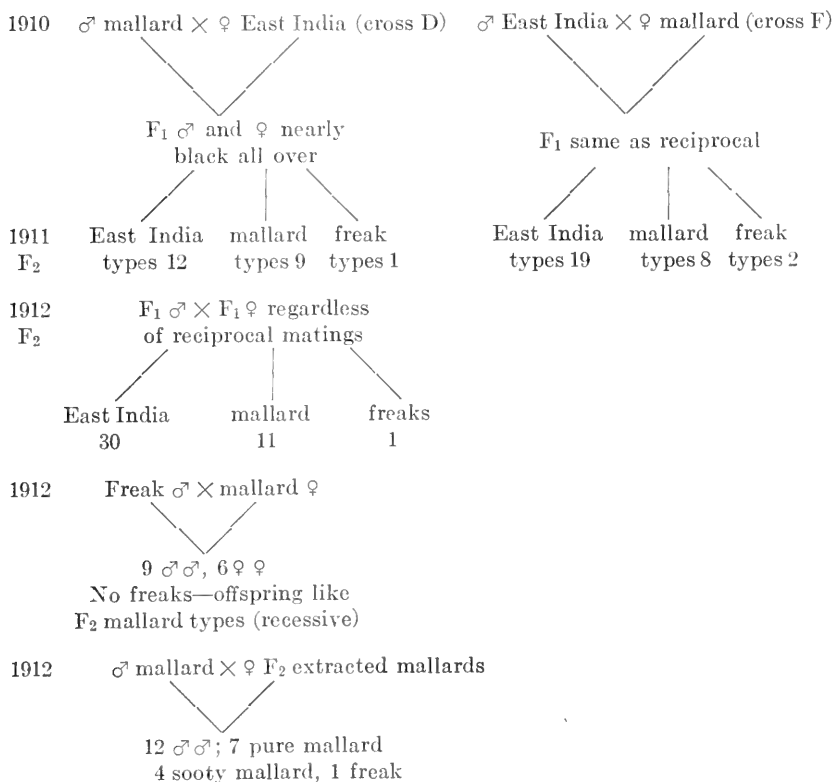
where the chestnut in the mallard ends. There is no relation between amount of blotching and general color of the bird. Some of the most mallard-like individuals are solid colored, while some of the blackest birds are heavily blotched. The mottling of the hind neck and post-ocular region, so common in the down plumage does not appear in the adults except very slightly in two cases. This diversity of the males is without doubt due to heterozygosis, but this point was not tested.

An examination of the recessive (mallard) class of F_2 's shows them to be all alike in down, being very close to the mallard ducklings, but always, or nearly always, with a slightly broader and darker ocular streak. On maturity these birds are pure mallards to the casual eye, but close inspection reveals the following points. The males, only four of which were reared, are usually to be distinguished by a very slight sooty appearance of the lower abdomen, which in one specimen, No. 411, is quite pronounced (pl. 6, fig. 3). Two of these males would certainly not be noticed as being in any way peculiar (pl. 6, fig. 4). The females, however, are all slightly 'contaminated' by the cross, as is seen from their darkened appearance, both above and below, and the very distinct sooty look to the lower abdomen (pl. 6, fig. 5). The third or freak class, which must also be regarded as recessive (see below) is always jet black in down, like the 'freak' mallards described in the 'freak mallard' experiment. There are three males and a female. The males are darker on the back than the pure freak males, and one of them, No. 160, 1911, has a little area of chestnut below the neck-ring. The appearance of this slightly contaminated freak type is not easy to explain. One of these freak males (No. 207, 1911) was tested in 1912 with the mallard stock (cross I, 1912), and produced nine males and six females. There were among these no freak types. In down plumage all were of mallard coloring with somewhat darker ocular stripes, like the recessive mallards from the F_2 East India cross. When adult three of these males were not to be told from mallards, while six had the slightly sooty abdomen. The females were darker than mallards and many had the anterior white wing bar reduced or absent. In

fact the birds of this lot are just like F_2 recessives, and the freak type has been entirely dominated. This shows that this type must be classed as a recessive, and probably was introduced into the cross as a recessive character from the mallard side.

The dominant F_2 's need not be further considered, as they were not tested. It was desired to test the contaminated recessive female F_2 's, and two of them (Nos. 202, 184; 1911) were mated to a male mallard (cross M, 1912). From this cross there resulted twelve males. Seven of them are classed as pure mallard type and four of them as sooty mallard type (sooty on lower abdomen, sometimes slightly darker above, anterior wing

TABLE 2

Scheme of matings in Black East India \times mallard experiment

bar sometimes reduced). The remaining male is a pure freak type and was jet black in the down, as this type always is (♂ 455). The females were not all carefully examined, as they were discarded for another purpose. The only specimen saved is practically a pure mallard with the wing bars both present. The 'contaminated' females of the F_2 recessives therefore passed on to about half of their male offspring, the contamination character (sooty abdomen, etc.), but the peculiar freak type also appeared again in one case.

FREAK MALLARD EXPERIMENT

In the spring of 1909 one hundred and twenty-five mallard eggs were purchased from George Edgar, gamekeeper of Tranquility game preserve in Connecticut. From this lot sixty-five birds were reared to maturity, forming the basis of mallard stock described elsewhere.

At hatching time two of these ducklings appeared so remarkable in color that they were banded. They were coal black. One of them died, but the other, ♂ No. 39-256, matured, and was found in October 1909, to represent a new type of mallard, a clear-cut variation, which I have called 'freak mallard' (pl. 5, fig. 3). This male can be described in a few words. There is entire absence of white neck collar and chestnut breast area, the silver color of the lower parts extending up to the green of the neck, while the speculum is dull black instead of metallic green. No other duckling in this lot showed any departure from normal.

In 1910 ♂ 39-256 was placed in a mating pen with a normal ♀ mallard from the original lot, and seven ducks were reared to maturity, ♂♂ Nos. 116 and 145 and ♀♀ Nos. 114, 117, 93, 115, 118. They were all normal mallards, the freak character proving entirely recessive.

In 1911 one of the F_1 males and two F_1 females were mated (cross E, 1911) and there resulted five black 'freak' ducklings and seven normal ones (pl. 5, figs. 5 and 6). Only two of the freaks lived and these were both females, but when adult they were seen to be different from mallard females. They are always to be

told by absence of the dark ocular streak and the light supra-ocular streak, and presence of a dark, streaked throat, which in the normal bird is plain buff color. The throat in the female freak is exactly like its cheeks (pl. 5, fig. 4). These 'freak' mallards appeared to lack vigor and were by no means easy to rear.

In 1912 ♀♀ Nos. 221 and 219 were mated with the original freak ♂ (cross N, 1912), and there were reared five birds, three males and two females, all typical freaks, showing that the freak character had segregated in a pure state. In 1913 this generation gave rise to still another one (cross R, 1913) all similar in character.

It is interesting to note that out of thirteen freak ducklings hatched in 1913, only seven were reared. Two female freaks in 1913 laid only forty-one eggs. Twenty of these were set, but only thirteen hatched. The English stock mallards on the other hand lay about forty eggs each, and these eggs are 80 to 100 per cent fertile.

I have failed to find any evidence of this 'mutation' occurring in wild killed birds. Dewar and Finn (6) mention its occurrence in domestic mallards, and Rogeron (27) reared many of them. Rogeron thought that he obtained his freaks from pure wild stock, but of this I think there may be some question, because the breeding European wild stock seems to be rather strongly given to variations and contaminations, as Rogeron's own book shows. It is, however, possible that this variation does occur in European wild mallards; it certainly does not in American wild killed birds. This freak variation is common in the ducks of the London parks, as I have noticed myself.

Besides the characters above-mentioned, there is often a reduction of the white speculum bars in the female, and generally a darker look all over in that sex. The first juvenile plumage of both sexes is said by Rogeron to be more uniform, a point which I did not notice. The downy young are always black all over, including the bill, legs, and feet.

This is an exceedingly clear-cut mutation, and has showed no tendency to vary in my stock. Finn (7) mentions seeing gray-

breasted birds with a very little chestnut at the base of the neck, but no such intergrade has appeared here.

This freak condition may be of the same nature as the so-called *Chrysolophus obscurus*, the dark form of the golden pheasant, which was at one time thought to be a new species. It apparently segregates out clearly in aviary strains of golden pheasants but is never found in the wild.

We thus see that although the chestnut breast and white neck ring of the mallard are inherited in a very complex way when crossed with other species, there nevertheless exists some 'modifier' or other single factor whose presence is necessary for their expression. Also we have every reason to believe that the loss of this 'modifier' is associated with relative sterility and lack of vigor.

TABLE 3

Matings in 'Freak Mallard' experiment

English mallards, about 500, including three freaks

♂ freak No. 39-256 × ♀ mallard (from mallard stock)

F₁.....2 ♂♂'s; 5 ♀♀'s (normal mallards)

F₂.....7 normal mallards; 5 freaks

♂ freak No. 39-256 × ♀ F₂ freaks

3 ♂♂, 2 ♀♀, all freaks

4 ♂♂, 3 ♀♀, all freaks

Prince of Wales (Phasianus principalis) crossed with ring-neck pheasant (P. torquatus)

This cross was started with the idea of testing the inheritance of the white neck-ring of *torquatus*. All the true pheasants (4) may be roughly divided into four groups, according as they have, or have not, a white neck ring, or according to whether they fall into the green rumped or the red rumped group. By

far the largest group of these four possible combinations is the red rumped dark-neck (no neck ring) group; and into this falls *principalis*. This group contains 9 species and 5 sub-species. The next group, the combination of red rump and white collar, is a very small one. It contains but 2 species and 1 sub-species. The green, gray, or slaty rumped group with dark necks number 8 species and 2 sub-species, while lastly the green rumped and white necked group contains 4 species and 3 sub-species. There may be said to be therefore a tendency in nature towards a combination of red rumps and dark necks and green rumps and white neck rings. It was thought of interest to test the question whether an association of these characters would be found in hybrids. It has been shown above that in duck hybrids, for instance, the mallard characters, green head and chestnut breast, are always correlated in their intensity.

The striking features about the *principalis*, or Prince of Wales, pheasant are as follows: Neck ring absent, lesser and median wing coverts white, with white shaft stripes on the greater coverts; rump and upper tail coverts orange red with a few fine black dots; tail barring reduced to faint lines. *Torquatus* or ring-necked pheasant: White neck ring well marked, though absent ventrally; rump greenish to greenish slate, with sub-terminal bars of brilliant green; lesser and median wing coverts mostly sandy buff color; tail barring very marked, especially towards the tip, where black areas from 6 to 12 mm. wide occur. There are other differences between these species, especially in the mantle, in the flanks, and in the metallic color of chin and throat, but they are characters rather too subtle for the present study.

In 1909 a pair of pure wild *principalis* pheasants were secured from Wallace Evans, Oak Park, Illinois. The male was carefully compared with a wild killed skin from Merv, Trans-Caspian, Asia, collected in June, 1889 (pl. 7, fig. 1). The two birds agreed very well, and there was only one slight difference, the tail bars on my live specimen being fainter than those of the Merv skin (a *principalis* character). The rump also is redder in my skin, but taken as a whole the differences between the two birds are no more than individual or seasonal.

The ring-necks or *torquatus* used in this cross belong to a strain from which at least two hundred individuals have been reared on the farm. In no case was the white neck ring absent (pl. 6, fig. 2).

From the original pair of *principalis* there were reared in 1912 and 1913, thirty-three pure bred offspring, none of which showed any departure from type.

In 1910 the original male *principalis* was mated with two females from my *torquatus* stock. From this pen I reared four males and five females, which may be described as follows (pl. 6, fig. 3). Males: neck ring reduced but present; wing coverts grayish white, not so white as in *principalis*, white area less than in that species; rump rich reddish chestnut, not the gold red of *principalis* or the greenish slate of *torquatus*; tail barring very narrow, showing partial dominance of the *principalis* parent. In all other respects the hybrids are intermediate. The female is much lighter colored than the *torquatus* female, and quite near that of *principalis*.

In 1911 mating was made, with a trio of the F_1 generation and resulted in the rearing of ten F_2 males and a number of F_2 females. The latter were killed as soon as they could be sexed, on account of lack of room.

These F_2 males all had the neck ring, though in one case it was reduced to a few white feathers. They were graded in an arbitrary manner with relation to the four characters described above, neck-ring, wing-coverts, rump, and barring of tail. The result obtained is shown in table 4.

There is, as far as can be determined with small numbers, no correlation between any of the above characters. They are distributed regardless of individuals. For instance, the nearest approach to a *principalis* is specimen D, which has a small neck ring, a very red rump (much darker color, however, than *principalis*) white wing coverts, but the white patch reduced in area, tail barring intermediate. The mantle and the throat in this bird approach somewhat the *principalis* color pattern. To sum up the series, there is a very distinct segregation of the characters considered, but no clear dominants or recessives appear. Very

TABLE 4

White neck ring.....	trace	1 case
	small	2 cases
	intermediate	3 cases
	large	4 cases
Rump and upper tail coverts, color..	green, almost torquatus	2 cases
	reddish	2 cases
	greenish	2 cases
	rich red; about like F_1	4 cases
Wing coverts, color.....	brownish	4 cases
	intermediate	2 cases
	whitish	4 cases
Tail barring.....	feeble	1 case
	intermediate	6 cases
	strong	3 cases

large numbers would be of interest in this cross. The tendency to segregation is probably greater, than in, for instance, the pin-tail mallard duck hybrids (pl. 6, figs. 6-10).

In 1912 (cross E) the original *principalis* male was mated with two F_1 females and fifty-four chicks were hatched. Unfortunately a severe enteritis broke out among these birds before they were mature and only nine males were left available for study in November (pl. 6, figs. 4-5).

Comparing these back cross birds with F_2 birds the following generalizations are at once to be made. These nine three-quarter blood *principalis* are all to be told at sight from the ten F_2 by the very marked white wing coverts. The white area is whiter and of greater extent in all cases. There is no marked segregate, the lot is fairly uniform. The upper back and mantle is lighter in all cases except in No. 829, where it can be nearly matched with specimen F_2D . The neck ring runs from zero in No. 586 through two other specimens which have only one white feather each, to No. 835, in which there is a very narrow ring (about 5 mm.) present on sides only. The tails of these back crosses are all very narrowly barred, more so than in any F_2 , except in one case, No. 586, which matches F_2A . The rumps are all red; they are more golden red than the F_2 rumps, the least red, No. 582, matching exactly the reddest F_2 rump (spec. D).

There is then an approach, perhaps even an overlapping, of individual characters between the F_2 's and the three-quarter blood *principalis*, but this does not refer to individuals, which, all characters taken together, never approach each other in the two series. The uniformity of the back cross is interesting. It is like the uniformity of the three-quarter black ducks, three-quarter Australians, and the three-quarter blood mallards.

To anyone familiar with pheasants there will at once be a query, was the *torquatus* stock pure? This is a criticism which cannot be lightly disregarded. The English pheasant was, as is well known, originally pure *colchicus* from Asia Minor, a ringless pheasant with sandy brown wing coverts, red rump and upper tail coverts, and wide tail bars. Nearly pure specimens of *colchicus* may be even now occasionally seen in English markets, but they apparently all have the green terminal bar on the rump feathers, characteristic of *torquatus*. If the ring-neck contains blood of *colchicus* sufficient to effect an increased segregation in hybrids with other species, the neck ring and the rump color should be the regions most affected, for these are the most sharply contrasted in *colchicus* and *torquatus*. Such, however, can hardly be said to be the case. It is intended to test this point in crosses between *principalis* and other species, but for practical purposes the ring-neck can be considered a fixed race. Ghige has also reached the same conclusion.

To sum up: This cross gives intermediate hybrids with a tendency to a mild form of segregation in F_2 , this segregation occurring without apparent correlation of parental characters. The most extreme F_2 characters varying in the direction of *principalis* exactly meet the three-quarter blood *principalis* series. The three-quarter blood series is more uniform than the F_2 series, and does not overlap it. It is probable that three-quarter blood birds can always be told from F_2 birds, although some individual characters may be the same in the two series.

INHERITANCE OF SPOTTING AND ALBINISM IN PHEASANTS

In 1911 I received as a gift a male pheasant, of the ordinary *torquatus*, or English stock, a partially white male bird (pl. 8, fig. 1). The head is mottled with black, and the lower back, rump and wing coverts are very much mixed with white. The primary quills are mostly white. The lower parts except the abdomen, are nearly normal, while a part of the mantle is also nearly normal.

I mated this pied bird with my ring-neck stock in 1911, and reared one setting of eggs, all normal ring-necks (cross G, 1911). In 1912 and 1913 a trio of these F_1 's was mated to produce an F_2 generation. The results of the two years added together are as follows: 68 normal chicks; 22 white chicks. All chicks that died in the shell are included in these figures, which are of course a perfect Mendelian result. The original male died in September, 1911.

These white F_2 pheasants were very delicate and many died young. Only eight of the twenty-two were reared, though the greatest care was taken with them. Cronau (5) obtained white pheasants, and found white entirely recessive. He had great difficulty in rearing his white birds. Whites and partial whites crop out in many stocks of English (*torquatus*) pheasants, but are usually destroyed as being inferior for show or sport.

Now as to the coloring of this F_2 generation of whites: They run all the way from a bird about half white, as in 784, 1912, or 1178, 1913, to pure white birds with very slight brown ticking here and there, as in ♀ 780, 1912, and ♂ 781, 1912. The eyes are always a dirty bluish gray, some a little darker than others, but not at all the bright hazel brown of the normal bird. The legs and bill are very pale pinkish.

A selection was made from the whitest birds, and in 1913 a trio of them (all nearly immaculate) was mated (cross D). The mating resulted in an F_3 generation of eleven mature birds. They were all white, or nearly so. The brown ticking when present occupied a patch on the occiput, a few feathers in the wing coverts, or a little brown streaking of the flanks and breast

(pl. 8, fig. 2). One or two of the birds had a creamy appearance all over.

A selection has been made of the darkest F_2 birds, and it is hoped to rear large numbers, with the idea of seeing whether these pied recessives may by any possibility throw a dominant (normal) individual. It is of course always possible that inbreeding of this sort will end in the loss of the stock, as pheasants seem to be very susceptible to its dangers.

Summary: Spotting behaves in pheasants as a unit character. Selection of the whitest F_2 birds produces an F_3 generation which is far in advance of the F_2 's in 'whiteness,' though some regression occurs.

Lady Amherst (Chrysolophus amherstiae) and gold pheasant (Chrysolophus pictus) crosses

In this experiment, which was begun in 1911, I planned to make reciprocal crosses, carry them to F_2 and make all possible back crosses between the two F_1 generations and both sexes of both parents, in order to detect any trace of sex-linkage. This entailed twelve matings, rearing of the males until fourteen months or more of age (full plumage is not assumed till late in the second summer in either of the parent species) and inbreeding species made delicate already by many generations of aviary life. The greatest difficulty encountered, however, was the fighting propensity of the males, which were being held over for full plumage. This necessitated placing each male in a separate cage from early in the spring to at least the following August. This experiment has taken up much room, and progress has been extremely slow. There has been great mortality among hybrids; often as many as half or two-thirds of the young birds have died in a summer, probably on account of lack of space and crowding on soiled ground. However, nearly all the crosses have been carried out, though not all the birds are matured enough for study.

In regard to back crosses it is yet too early to speak, but the two series of F_2 present a feature that is so remarkable that it seems best to record the phenomena at this time.

There were in all a very good series of seventeen F_1 males raised from both reciprocal crosses, and these were found to be always alike. An excellent plate of this hybrid is given in Elliot's monograph of the Phasianidae. There is a slight amount of variation in the yellow area on the sides of the breast, representing the demarcation in the Amherst between the green breast and the white belly. All other features are constant so far as my observation goes. We need not take time here to describe this hybrid in detail.

There are now available for study nine F_2 ♂♂ from the cross ♂ Amherst \times ♀ gold, and seven F_2 ♂♂ from the cross ♂ gold \times ♀ Amherst.

The first series, with the Amherst for male parent, is far closer to the golden type than the companion series with gold for male parent. This apparently means that the male Amherst has passed on to his progeny less of his own male characters than the female Amherst has passed on to her progeny. It is also to be remarked that the ♂ gold and ♀ Amherst F_2 's are very close to the F_1 's, though more variable, while the other series is very much removed toward the golden type and also varies around its own mean. The two series about meet, but do not overlap in the least. The differences are marked ones, such as: color of mantle, color of crest, color of upper tail coverts, presence or absence of Amherst green on the upper breast, presence or absence of yellow area at breast-belly junction, etc. It is difficult to see how there could be any mistake in this experiment. The results appear to be clear-cut in every case. They might, however, be criticized on this score: that the male Amherst used in the cross contained golden blood, or that perhaps the ♀ gold contained a little Amherst blood. The most likely contamination is no doubt to be looked for in Amherst stock, as this is a rarer and more delicate species. But the golden blood, if present at all in the Amherst strain, must be very small in amount to escape close inspection, and it seems impossible that it could be responsible for the clear-cut difference described above. Gigli (17) notes that the aviary Amhersts have been developed by crossing with gold and back crossing with male Amhersts until all

golden blood was swamped. He himself crossed back to Amherst several times. In the second back crossing (gold blood $\frac{1}{8}$) the offspring, he says, are pure Amherst, except for slightly broken transverse bars on the tail and slightly dim tail patterns. In the fourth or fifth back crossing all trace of gold blood vanishes. The tail characters are the last to disappear.

I feel sure that if my stock specimens of Amherst contained golden blood, it was less than one-sixteenth in amount.

The best argument against the contamination theory is that if the male Amherst had had a dose of golden blood, his F_1 hybrids would have been different from those of the reciprocal cross. This has been found not to be the case. The F_1 and F_2 $\varnothing \varnothing$ are apparently all alike, but for lack of space they have been discarded early.

It is planned to test again the question of the unlike F_2 $\sigma \sigma$ with carefully inbred strain of the parent species, which are now available here.

TABLE 5

Matings described above

σ Amherst \times \varnothing Gold	σ Gold \times \varnothing Amherst
F ₂ 5 σ 's; 8 \varnothing 's	11 σ 's; 12 \varnothing 's
....similar....	
F ₂	F ₂
F ₂ 9 σ 's, about like $\frac{3}{4}$ blood golden and showing very little Amherst as a series, but some variation	7 σ 's, like F ₁ 's only a little more variable

OTHER WORK ON PHEASANTS AND DUCKS

It is now necessary to review some of the important features of the work of Alessandro Ghigi of Bologna. Professor Ghigi has made very extensive studies with hybrids of the pheasant genera *Gennaeus* and *Phasianus*. He also worked with the guinea-fowls and with fowls and pigeons. His early work was undertaken with the idea of getting at a more rational classification of the groups involved. He thought the present method

of classifying species upon secondary sex character of plumages was not natural, and he laid stress on the phylogenetic character of plumages in females and young birds. He also showed the importance of ecologic factors in determining fertility and sterility in hybrids, and he showed that there was as a rule a typical difference in the plumages of fertile and sterile hybrids among pheasants. In fertile hybrids the male characters were more or less intermediate between the parental types and tended to produce new and more or less stable hybrid forms. In sterile hybrids there was a distinct tendency to a reversion to the more primitive plumage of the female or young, or to the assumption of characters uncommon to either parent.

Many of Ghigi's conclusions are drawn from a study of the *Gennaeus* pheasants, a large genus of which, the silver pheasant, *G. nycthemerus*, is a typical and familiar example. A brief notice of his work is necessary, especially as this work has not been given the recognition which it deserves (13).

After a systematic survey of these pheasants, twenty-seven species of which are recognized (some of very doubtful value), he goes on to describe the various hybrid combinations which he obtained between the species *nycthemerus*, *horsfieldi*, *lineatus*, and *muthura*. He obtained besides F_1 's several back crosses and complicated hybrids combining three or four species. Some of these will be mentioned again in considering segregation of specific characters. He then goes on to compare the characters of these hybrids with the various wild species which are closely crowded into Burma, Southeastern China and the Shan states. The characters under consideration are very numerous and complex, but chief among them may be mentioned the mantle pattern, the color of the fringe on the lower back and rump, shaft markings on feathers of sides, pattern of middle tail feathers and color of legs. He recognizes fourteen more or less opposed characters in the males and nine in the females. From this consideration he takes the position that the *G. affinis* of Oates is a hybrid with blood of *G. horsfieldi* predominating, and containing a trace of *lineatus*. *G. andersoni* and *G. belli* can be interpreted as hybrids, near to *lineatus*, but with the red legs

of the silver pheasant, and so on. In all, ten species are accounted for in this way.

Ghigi next considers the distribution of the group, and shows that most of the recently described species occur in the region where the *horsfieldi*, *nycthemerus*, and *lineatus* converge toward central Burma. The species of the genus overlap very much more than the true pheasants, no two species of which are supposed to be found in the same region.

The last part of this work is composed of general considerations in regard to the dissociation of specific characters and a comparison between these characters and some of the ordinary racial characters seen in domestic products. In general it is maintained that crosses amongst systematic species (of phasianidae) where fertile hybrids are obtained, lead in a definite way to new stable forms in which characters prevailing in the parents are associated in a different way. He does not give this conception as in conflict with Mendelism, but regards the sum of specific characters as decomposable in several unities, which are destined to separate themselves in the successive generations after the first crossing. The species characters cannot often be classified as antagonistic pairs but as a complicated series often in correlation, but capable of being broken up so that in some cases pure characters appear. He would distinguish between those species which produce new hybrid combinations and retain their hybrid characters in further crosses, and those species which cannot produce new types in hybrid combinations, and always tend to assume parental forms when back crossed; in other words, Mendelian disjunction. For example, *leucomelanus* \times *horsfieldi* and *leucomelanus* \times *lineatus* produce intermediate forms, and on further crossing; new stable combinations, because the parents differ in their character complexes. On the other hand, *leucomelanus* \times *muthura* and *leucomelanus* \times *albocristatus* cannot give rise to new forms, for they differ in single undecomposable characters and their products tend to resume parental types. *Muthura* has a black fringe and a steel blue rump, *albocristatus* a whitish fringe and rump widely spotted with white; *leucomelanus* has the fringe of *muthura* and the rump of *albocristatus*, etc. Under this class also comes the Amherst-golden combination, for these

species differ in certain clean-cut antagonistic particulars and their back crosses tend quickly to resume parent types (17, p. 236). In other words, Ghigi, would consider the first category of parents as elementary species, and the second category as varieties. The really primitive species he considers as sterile or partially so, and always able to maintain themselves in the wild. It is obvious, however, that no such classification as this can be strictly enforced. No parental types (as far as the work of Ghigi, Mrs. Haig Thomas, and myself has shown) are ever entirely reached by the first back cross, at least not in the males, though it may be argued that in no species crosses of birds have numbers sufficient for such a result ever been reached. Furthermore, primitive species do not always produce sterile hybrids, as Mrs. Haig Thomas has shown for *nycthemerus* and *Swinhoii* (20).

We may now return to that part of Ghigi's work which most concerns us, the measure of 'variability' induced by crossing wild species. Ghigi himself did not pay much attention to this point, and did not attempt to raise large numbers in his pheasant crosses. For theoretical purposes it would have been interesting if he had restricted his efforts a little more to certain typical hybrids. In the work on the *Gennaeus* pheasants which we have been considering, among the list of hybrids we note that ♂ (*nycthemerus* × *muthura*) × ♀ *nycthemerus* produced two males half-way between the first hybrid and pure *nycthemerus*, but one of them had red legs. The female was indistinguishable from pure *nycthemerus*. ♂ *G. horsfieldi* × ♀ (*nycthemerus* × *horsfieldi*) produced four ♂'s and three ♀'s. One ♂ was like *horsfieldi* except the white fringe on his back was narrow, and the tail was nearly black. The second differed in having brown spots on the external margin of the first ten secondaries, hardly any white fringe, middle tail feathers striated with white and brown, and with other differences (specimen not entirely developed). The third had a tail like the last, but white stripes on several feathers of the sides of the neck, a large white band on feathers of rump and remaining parts like No. 1. In the fourth male there appear to be several distinct differences: Crest shining black and nuchal area black; lower parts blue-black

like crest; back black with a few white stripes; scapulars and wing coverts black with three or four pairs of pure white stripes; middle tail feathers vermiculated on internal web, white on base, etc. Two of the females of this cross show variation, and one is the same as pure *horsfieldi*.

In cross

$$\frac{\sigma^7 (\text{nyc.} \times \text{muthura}) \times \text{nyc.}}{\text{♀} (\text{nyc.} \times \text{horsfieldi})}$$

there were two males and two females. The males differed in the period of the assumption of the secondary sex characters. One of the females had red legs. In cross

$$\frac{\sigma^7 \text{lineatus} \times \text{♀} (\text{nyc.} \times \text{muthura})}{\text{♀} \text{horsfieldi}}$$

there were two males and four females and both sexes showed numerous differences. In cross

$$\frac{\sigma^7 (\text{lineatus} \times \text{muthura})}{\text{♀} (\text{nyc.} \times \text{muthura}) \times \text{lineatus}}$$

there were four males and two females; two of the males were the same and the other two differed, one being delicately striated above; females alike.

In considering these hybrids it is very essential to recognize the delayed secondary sex plumage which I myself have often seen, and which Ghigi mentions in several places. If this is not taken into account, variations will often be overestimated. This precaution Ghigi has apparently understood. The best case of segregation is found in a recent publication of Ghigi (17), which considers the cross $\sigma^7 \text{horsfieldi} \times \text{♀} (\sigma^7 \text{nyc.} \times \text{♀} \text{horsfieldi})$. He had twelve males; six of them were almost like *horsfieldi*, but differing a little from that species; the other six hybrids are variable and form a series from the F_1 hybrid type to nearly pure types, black like *horsfieldi* but striated on the back, wings and tail. This last result he considers at variance with much of his other work, but it is clearly a case of segregation, more pronounced than any obtained in my experiments.

On the guinea-fowls Ghigi's work is closely parallel to that on the *Gennaesus* pheasants (15). He concludes that of the seven-

teen species and sub-species, only about half are to be considered as the products of change or variation. The rest are forms derived from natural cross-matings.

With the true pheasants Ghigi has traced the geographical range of certain characters, neck ring, red or green rumps, etc. (18, p. 69), and speaks of the true pheasants as reducible into certain well-defined groups. As evidence of the stability of hybrid forms he mentions the hybrid combinations in hunting preserves. For instance, in parts of England the versicolor and colchicus has resulted in a green-backed pheasant, on the Continent combinations of versicolor and torquatus have resulted in a stable variety, the green, ring-necked pheasant. Other stable types of introduced pheasants can be readily adduced. The ring neck of Massachusetts shows very little variation, and yet it has a hybrid origin.

In working with pigeons and with the wild gallinae (a cross was made between a ♂ sonnerats jungle fowl and a series of farm-yard hens) Ghigi reaches the conclusion that both pigeons and fowls have had a polyphyletic wild origin. In pigeons, leuconotis, rupestris, and livia; in fowls, sonnerati, laffayettii and Gallus gallus (14) (17).

Ghigi has described a very interesting mutation from a pure strain of Swinhoe pheasant (11), which he believes to be the 'door' of a new variety. He also cites the so-called Bohemian pheasant as a mutant from colchicus (mentioned by Tegtmeier) and other indications of spontaneous change in this group.

In his later publications Ghigi is inclined to include hybridism as a direct stimulation to actual change (18, p. 84). For instance, when the sonnerat's jungle fowl ♂ is crossed with ordinary hens of the farm-yard, the scaly and barred wing coverts of the wild male vanish and do not reappear in further crosses. Such a character is held recessive, but Ghigi thinks it may perhaps manifest itself as a stimulus to change.

As far as sex-limited characters are concerned, Ghigi has not reported on reciprocal crosses. In his pheasant crosses (particularly the back crosses) he finds no real evidence of paternal or maternal influences and in all crosses where a certain domi-

nance is apparent he prefers to attribute it to a species character rather than to unequal sex transmission (13, p. 39). This would appear from my work to be the proper view, except that I have found an exception in the unlike reciprocal F_2 Amherst \times Gold crosses and in the sterile reciprocal hybrids Reeves \times torquatus (26).

Mrs. Haig-Thomas found (20) that in crossing ♀ silver (nycthemerus) pheasants with ♂ Swinhoe that in the F_1 's crossed back to the ♂ Swinhoe, the ♀ Swinhoe plumage appeared in a pure state. Also in this back cross she obtained a pure ♂ Swinhoe and three hybrid or impure ♂ Swinhoes. In other words, there seems to have been some variation among the males. She concluded that the ♂ Swinhoe was a heterozygote for sex.

It is interesting to note that Ghigi found silver \times Swinhoe hybrids always sterile (9), though the females laid eggs. My own F_1 hybrids from this cross are thus far partly sterile.

In another experiment Mrs. Haig-Thomas crossed (21) ♂ versicolor and ♀ formosans. She then back-crossed one of the ♀ hybrids to a ♂ versicolor, and obtained five females and two males. All the females were alike and very close to versicolor. The males (only two) showed some formosan characters. This was the same result as in the previous Swinhoe-silver cross.

Recently (22) the same writer has described further her work with the progeny of a cross ♂ Swinhoe \times ♀ silver pheasant. She mated an F_1 ♀ back to a silver ♂ and one of the resulting females was again mated back to silver. In this generation of seven-eighths silver blood there appeared two birds, a male and a female which showed new characters and were called mutations. This pair was mated and produced another lot of ten birds somewhat like their parents. This paper is confined almost entirely to the plumage of the female hybrids.

It may be remarked that the elements of this cross are very numerous. First, both the males and the females of the parent species differ widely. Second, the males assume adult plumage only in the second year and have a distinct primary plumage unlike the female this being especially marked in the case of the

Swinhoe. Thus we have six different plumages and their transitions to consider. Furthermore, although Mrs. Haig-Thomas does not appear to have encountered it, partial or complete sterility occurs in these hybrids and if present would without doubt effect the adult plumages (retard them).

The following points only can be noted. No pure silvers were obtained, either male or female, in the second back cross to silver male (seven-eighths silver). Female Swinhoe characters were transmitted by the Swinhoe male through four generations without addition of Swinhoe blood. The so-called mutants of the third generation produced offspring mostly like themselves and the females were different from any others in the whole series. There was, however, some sort of segregation among these ten birds and traces of Swinhoe parentage could be traced.

Mrs. Haig-Thomas finds a condition in some of these hybrids which she calls 'pattern transference' the transference supposedly of certain markings from one area to another or even from one sex to another sex. For instance, a pattern on the breast of one of the female hybrids looked like the flank pattern of the young male silver in transition plumage. The evidence for this interchange of patterns between the sexes seems hardly strong enough in this case, for the possibilities of variation and reversion are too great to be ignored.

Evidence is brought forward of two distinct breast and tail patterns in the females of silver pheasants which seem to occur both in aviary and wild shot birds. It is not a clear-cut dimorphism, but it might very easily further complicate this cross.

Among ducks there is a very large number of described hybrids, some sterile, others fertile, but very little genetic work has been done on wild forms. In 1905 (2) J. L. Bonhote reported upon a series of complicated matings between the mallard, the pintail, the spot bill, the Australian duck and Meller's duck. Although it is impossible to deduce any genetic facts from these matings, there are some interesting points in this work. In the second and third generations of Bonhote's 'trigen' and 'tetragen' combinations there was a distinct tendency towards certain types, and never variation enough to obscure the type to which the hybrid

belonged. Moreover, there occurred a sort of dimorphism in two cases (a dark and a light variety) which deserves notice. Two crosses were made as follows:

$$\begin{array}{l} (\text{mallard} \times \text{spot bill}) \times (\text{pintail} \times \text{mallard}) \\ (\text{pintail} \times \text{mallard}) \times [(\text{mallard} \times \text{spotbill}) \times \text{mallard}] \end{array}$$

The results in these two crosses and in their offspring bred together seem to be the same. Both of them give rise to a light and dark form which is always apparent in infancy and affects both sexes. The dark form has more mallard characters, the light form more pintail. Inbreeding has only been possible with the light form, which has produced in the third generation a very light-colored bird looking like the eclipse plumage of the first generation. Mallard characters are still present in the color of the chest and in the curly sex feathers of the tail. This third generation was very weak, and probably some of its 'eclipse' characters was due to partial or complete sterility. The tendency for these hybrids to vary along certain definite lines is of interest in considering the theory of Ghigi in dealing with complex species hybrids; that is, that there is present in species a tendency to certain *definite stable hybrid combinations*, not a mere shuffling of characters.

Another of Bonhote's crosses was the mallard-spot-bill-Australian hybrid. The drakes fall into two classes, differing in the amount of mallard which they contain, and the second generation male hybrids lose some of their male secondary sex characters as in the last case.

In 1909 (3) Bonhote described a remarkable duck, almost pure mallard, which originated from a fifth generation complex of spot-bill, pintail, mallard and Australian. This bird contained 50 per cent Australian, 25 per cent mallard, $12\frac{1}{2}$ per cent pintail, and $12\frac{1}{2}$ per cent spotbill. The only difference between this bird and a pure mallard was said to be the rather more defined and pintail-like vermiculations on the flank feathers. Although this certainly looks like a very remarkable case of segregation, it must be noticed that both the Australian and the spotbill blood, representing $62\frac{1}{2}$ per cent, are of more or less neutral effect;

that is, they are both dark, plain-colored species with no secondary sex characters.

Goodale's work on duck hybrids need not concern us, as it dealt with domestic varieties. However, Goodale found that in castrating domestic ducks with mallard plumage the males retained their full plumage, but lost the power of assuming the summer or 'eclipse' plumage (19). The castrated female gradually assumes a more or less complete male dress. He suggested that the female duck owed her plain color to some modifying element which prevents development of male color, and also that this modifier may be sometimes responsible for sex limited inheritance.

As is well known, cases of females assuming male plumage are very common in domestic ducks, in pheasants, and in other game birds. Apparently it is always due to sterility or disease of ovaries. The converse, however, is very rare. Geoffrey Smith and Mrs. Haig-Thomas (28) reach the conclusion that assumption of hen's plumage by the cock is rare and happens independent of disease or degeneration of the testes, being more in the nature of a transfer of sex characters.

In my own aviaries I once had a pure golden pheasant which had a very remarkable plumage. This bird was given to me, and lived only a short time, so that I do not know its pedigree. In appearance it seemed to have a very much exaggerated youthful male plumage, and looked entirely different from any stage of normal male or female plumage. It showed no evidence of hybridism. On dissection no trace of any sex organs could be detected in this bird, although unfortunately the abdomen was not in good condition at the time of post mortem.

GENERAL DISCUSSION

In studying birds we are dealing with characters often of quite fundamental systematic importance. Color and pattern in birds, at least in wild species, are known to be a satisfactory basis for classification. In mammals color is notoriously unreliable. The present study has been almost wholly an investigation into the inheritance of male secondary sex-characters.

In domestic birds the studies of Davenport and others have demonstrated a number of clearly Mendelizing characters. Sex-linked characters have also been described in canaries, pigeons, and fowls.

In our study of wild forms we have found, however, a very different state of things. We have seen that characters often apparently clear-cut and antagonistic do not segregate clearly. For instance, in the cross of golden with Amherst pheasants (not fully described here) the F_1 has a mantle color practically pure Amherst although the pattern is intermediate. In F_2 and in back crosses to both parents, this mantle color shows every possible gradation from the Amherst pure white, to the almost pure orange-red of the golden pheasant. There are no classes as might be expected from the dominance in F_1 . Again, in the F_1 's the reticulated tail pattern of the golden pheasant is nearly dominant, but there follows very little segregation, and even in back crosses to Amherst this pattern of the golden persists in a tenacious manner up to crosses containing only one-sixteenth or one-thirty-secondth of gold blood, as Ghigi has shown.

There is some evidence that in closely related geographical races there is a nearer approach to orthodox Mendelism, but this is never reached, even in back crosses, except occasionally in isolated characters or in the more undifferentiated plumages of the female sex. In species with unrelated character complexes there are only slight tendencies to a greater variation in F_2 than in F_1 , as is shown in the pintail \times mallard cross, or the Australian \times mallard cross. Such variation, comparable to that seen in size characters, may be explained as the manifestation of factor complexes, outwardly expressed in plumage characters, but not by any means necessarily specific units for these epidermal structures alone.

We have found in the above crosses only two clear-cut characters, that of the 'freak' mallard and that of the 'East India' duck. Neither of these segregating units is in any sense a species character, but closely resembles a large class of variations in domestic mammals and birds.

It is almost certain that the ordinary sub-species of the ornithologist is very far from being a unit variation.

Sex-linked inheritance is probably a feature of domestic races in birds. In wild species thus far examined there is no clear evidence of unequal transmission by the sexes. Both sexes can carry the characters of the opposite sex through several generations without an additional 'dose' of the character in question. The case of the unequal F_2 generations in the reciprocal gold \times Amherst cross is as yet unexplained and needs to be further verified. The difference in the sterile reciprocal Reeves \times ring-neck cross (25) can probably be explained on other grounds than unequal sex transmission of parental plumages.

A study of species hybrids in birds will satisfy any one that in almost every feather region the minutest details of feather pattern and color show the influence of both parental races. Only, in sterile hybrids, or hybrids between distantly related forms, do we find hybrids that are at all puzzling in appearance, as Ghigi has so often pointed out. In such hybrids we must often look for similarities in the youthful, female, or primitive plumages.

In crossing two species, only one of which is sex dimorphic, a more primitive type of male plumage is seen in the hybrids and in the back crosses.

In the mallard, a condition closely resembling eclipse or summer plumage is brought out by crosses with the black duck.

Far larger numbers than have yet been attained would be of great interest in selected species crosses in birds.

I wish to thank Prof. W. E. Castle for his kind assistance in preparing this paper.

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Pintail \times mallard experiment.

- 1 Pintail duck σ . 2 Pintail duck φ .
- 3 Mallard σ . 4 Mallard φ .
- 5 σ [(F₁ pintail \times mallard) \times pintail] showing mallard characters in lessened black scapular patch, shorter central tail feathers, shorter neck, etc.
- 6 φ [(F₁ pintail \times mallard) \times pintail] shows a bird very close to pure pintail φ , but having some mallard characters.

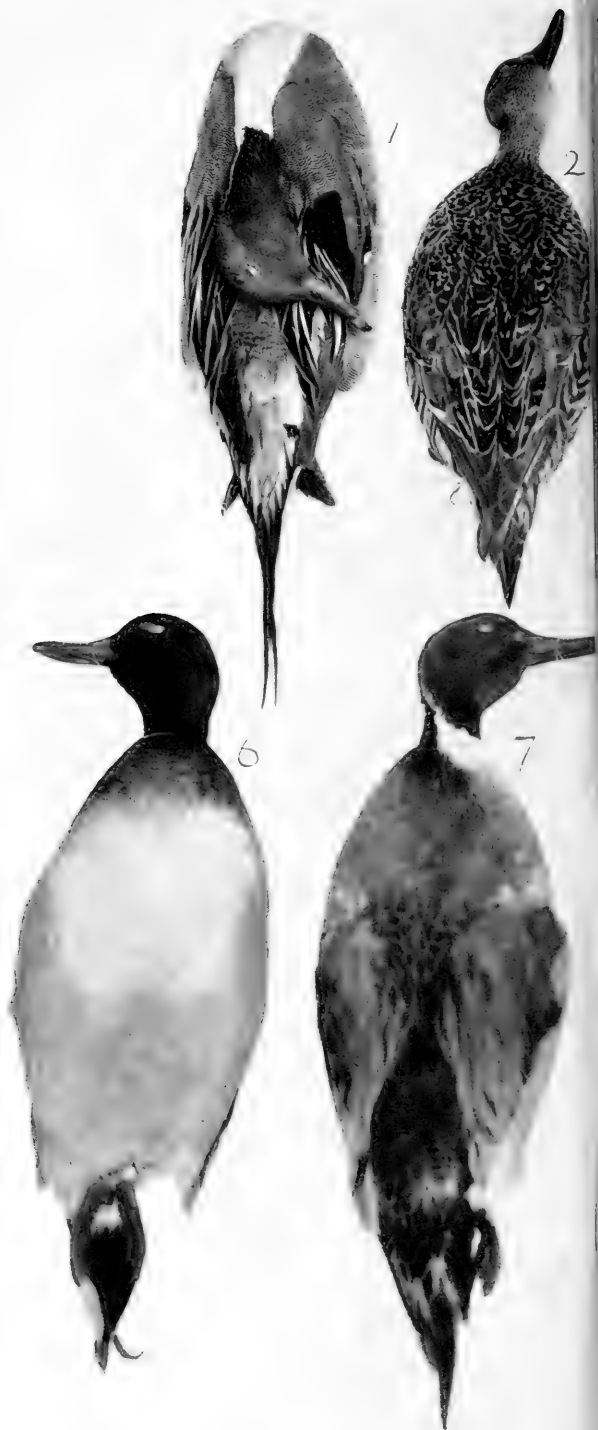


to 9 F_1 hybrids from σ^7 pintail and φ mallard. Figure 8 represents type F_1 males, No. 966; figure 7 is extreme variation in the direction of mallard S53; figure 9 is extreme variation towards pintail duck No. 849.

F_2 generation σ^7 , showing most extreme mallard variation No. 530.

F_2 generation σ^7 , showing most marked pintail variation, No. 525. The difference in the color of the heads between figures 10 and 11 does not show in the photograph.





Pintail \times mallard experiment.
1 to 7 Same specimens as in plate 1, but mallards left out.
1 Pintail duck σ . 2 Pintail duck ϕ .
3 to 5 F_1 $\sigma\sigma$ showing full extent of variation in F_1 .



6 F₂ ♂ No. 530.

7 F₂ ♂ No. 525 (dorsal view).

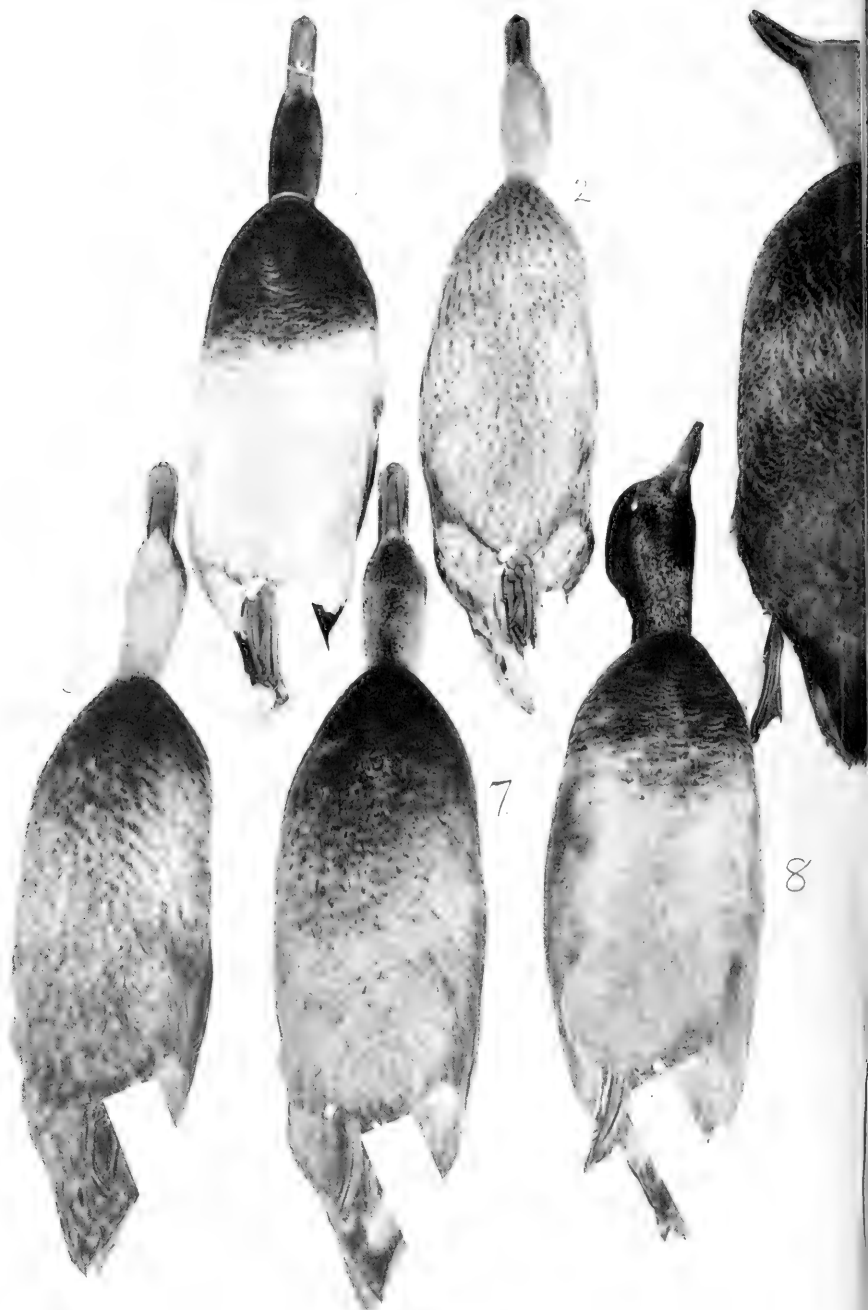
8 ♀ [(F₁ pintail × mallard) × pintail] ventral view.

9 ♂ [(F₁ pintail × mallard) × pintail] side view.



HYBRIDIZATION AMONG DUCKS AND PHEASANTS

JOHN C. PHILLIPS



Mallard \times black duck experiment.

1 Mallard σ . 2 Mallard ϕ . 3 Black duck (*a. tristis*) male or female.

4 F_1 σ from cross σ black \times ϕ mallard (call).

5 F_1 ϕ from cross σ black \times ϕ mallard (call).

6 to 8 σ F_2 types. 6 σ No. 61, type of F_2 generation. 7 σ No. 37 v
ate towards mallard, showing blackish chin, dark breast, black under tail coverts,



- 8 ♂ No. 174, *marked* variate in direction of mallard, having nearly solid green head and a chestnut breast, but the latter poorly marked off posteriorly, etc.
- 9 ♂ From $F_1 \text{ } \text{♀} \times \text{♂}$ black duck—very close to black duck, but always to be told by pattern on breast feathers (pl. 9, fig. 9).
- 10 ♂ From $F_1 \text{ } \text{♀} \times \text{mallard } \text{♂}$, same as $F_1 \text{ } \text{♂} \times \text{mallard } \text{♀}$.
- 11 ♀ From $F_1 \text{ } \text{♀} \times \text{mallard } \text{♂}$, same as $F_1 \text{ } \text{♂} \times \text{mallard } \text{♀}$.







Australian \times mallard experiment.

1 Australian duck, *A. superciliosa* (male or female).

2 Mallard

3 Type of F_1 ♂♂, specimen No. 77.

4 Type of F_1 ♀♀, specimen No. 78.



7 F₂ ♂♂ showing range of variation.
 ♂ showing very slight variation in mallard direction. No. 694.
 type of F₂ generation, No. 225. 7 A ♂ with dark uniform lower parts, No. 304.

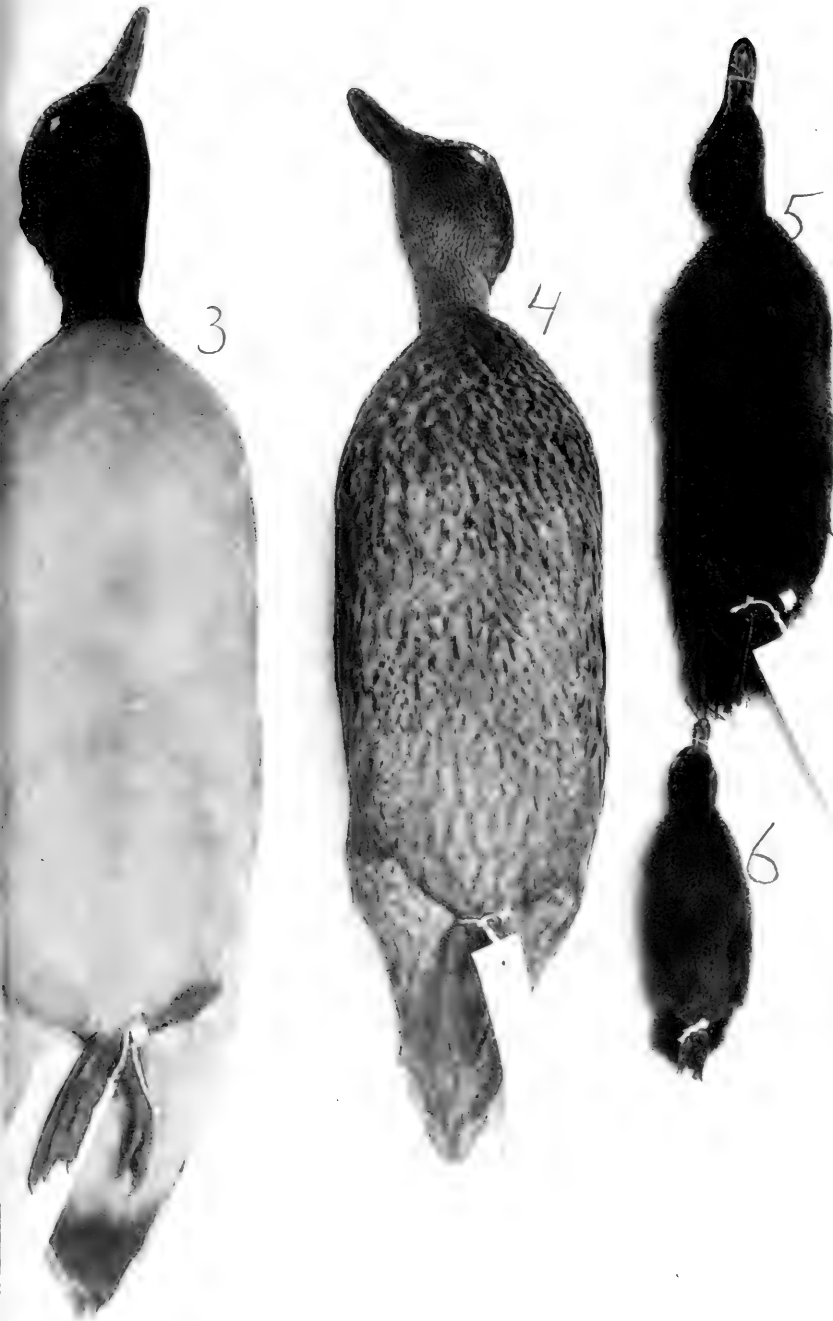


HYBRIDIZATION AMONG DUCKS AND PHEASANTS
JOHN C. PHILLIPS



Freak mallard experiment.

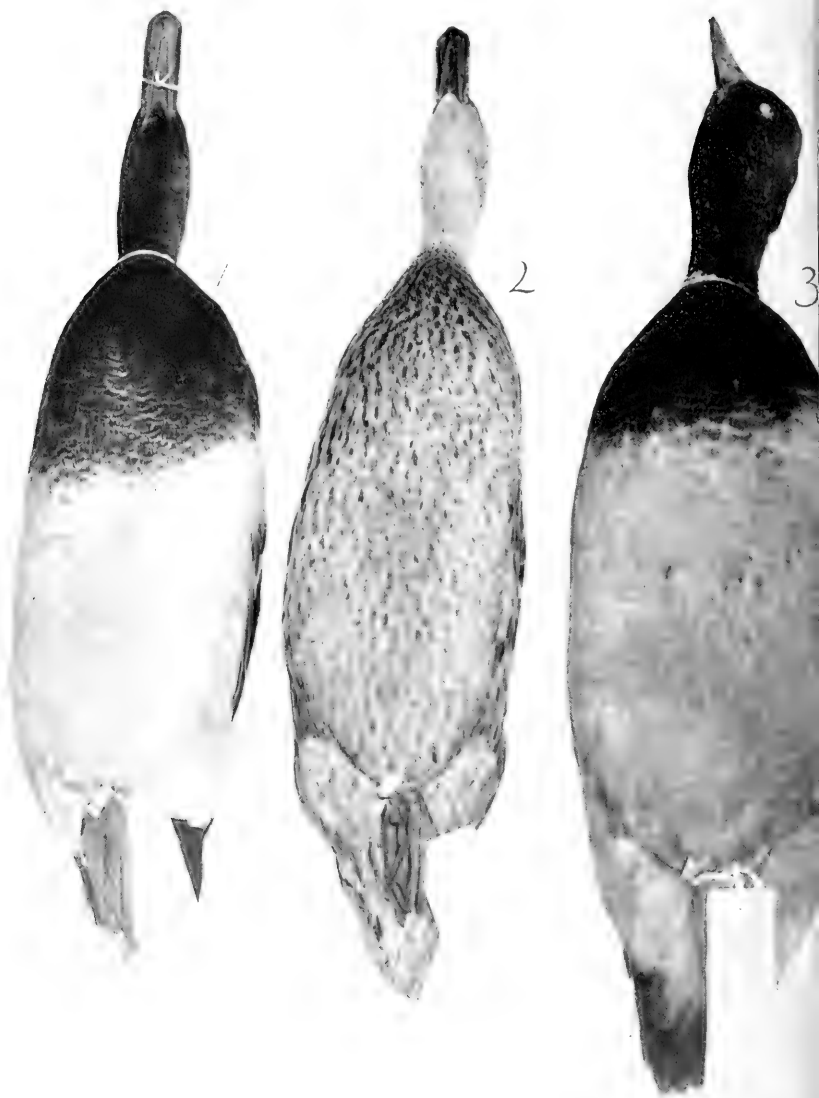
- 1 Mallard ♂. 2 Mallard ♀.
3 Original male freak, ♂ No. 39-256.



4 Extracted F_2 ♀ freak No. 219. The darkness of this bird is slightly exaggerated in the plate.

5 to 6 Young extracted freaks in down plumage, either sex.

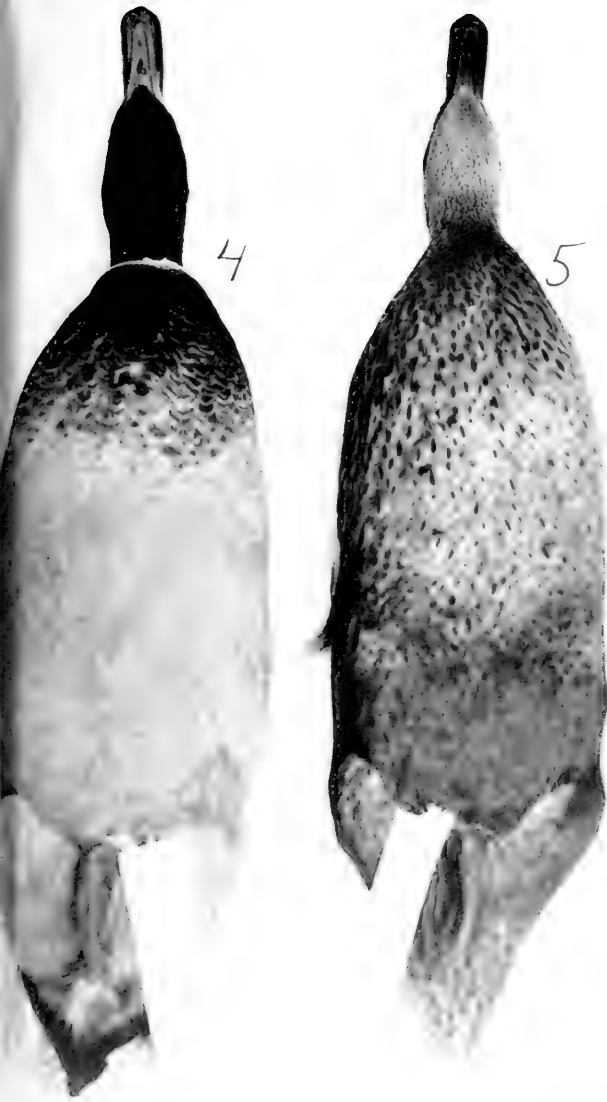
HYBRIDIZATION AMONG DUCKS AND PHEASANTS
JOHN C. PHILLIPS



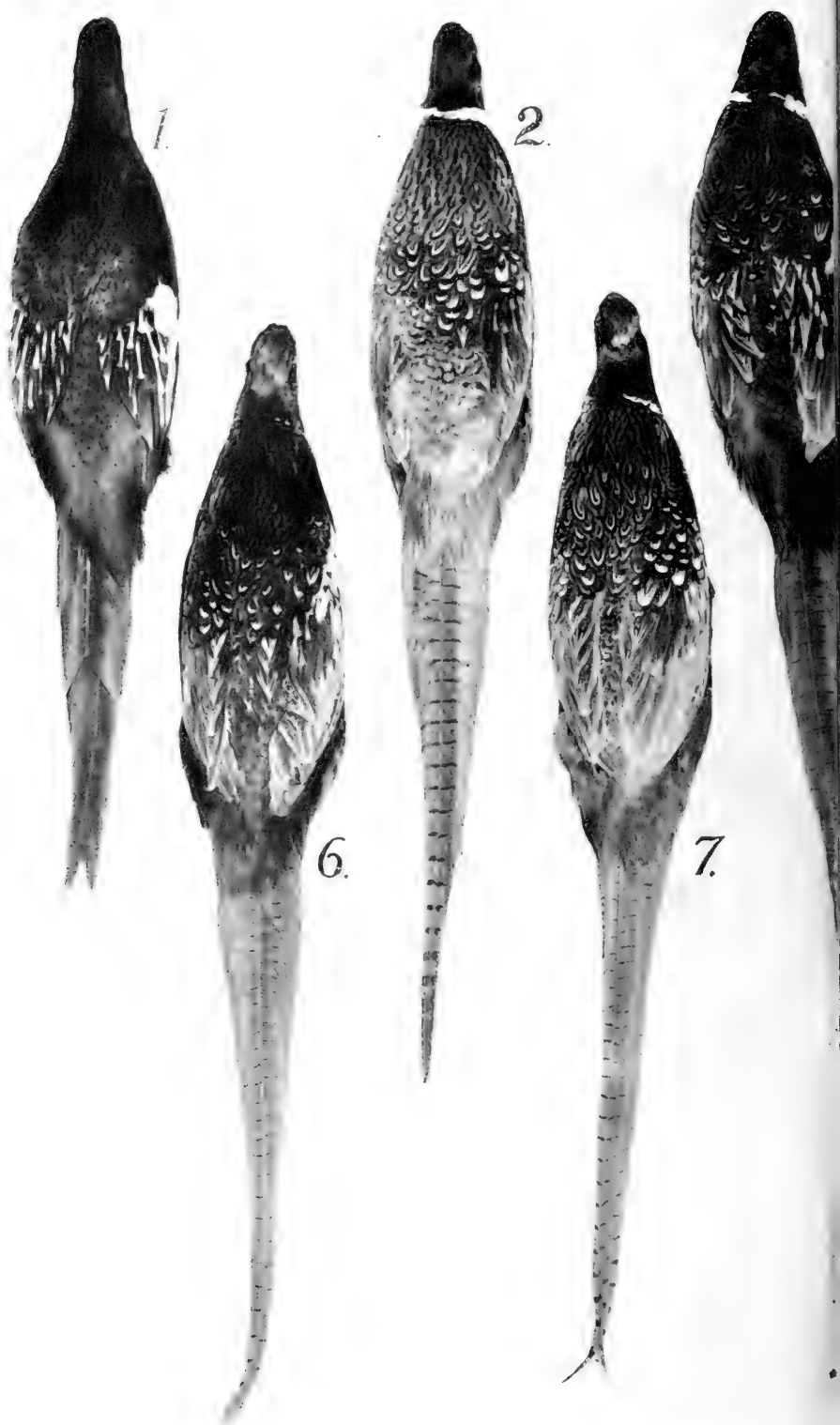
Mallard \times East Indian experiment.

1 Mallard σ^7 . 2 Mallard q .

3 Extracted F_2 mallard male dark type (contaminated).

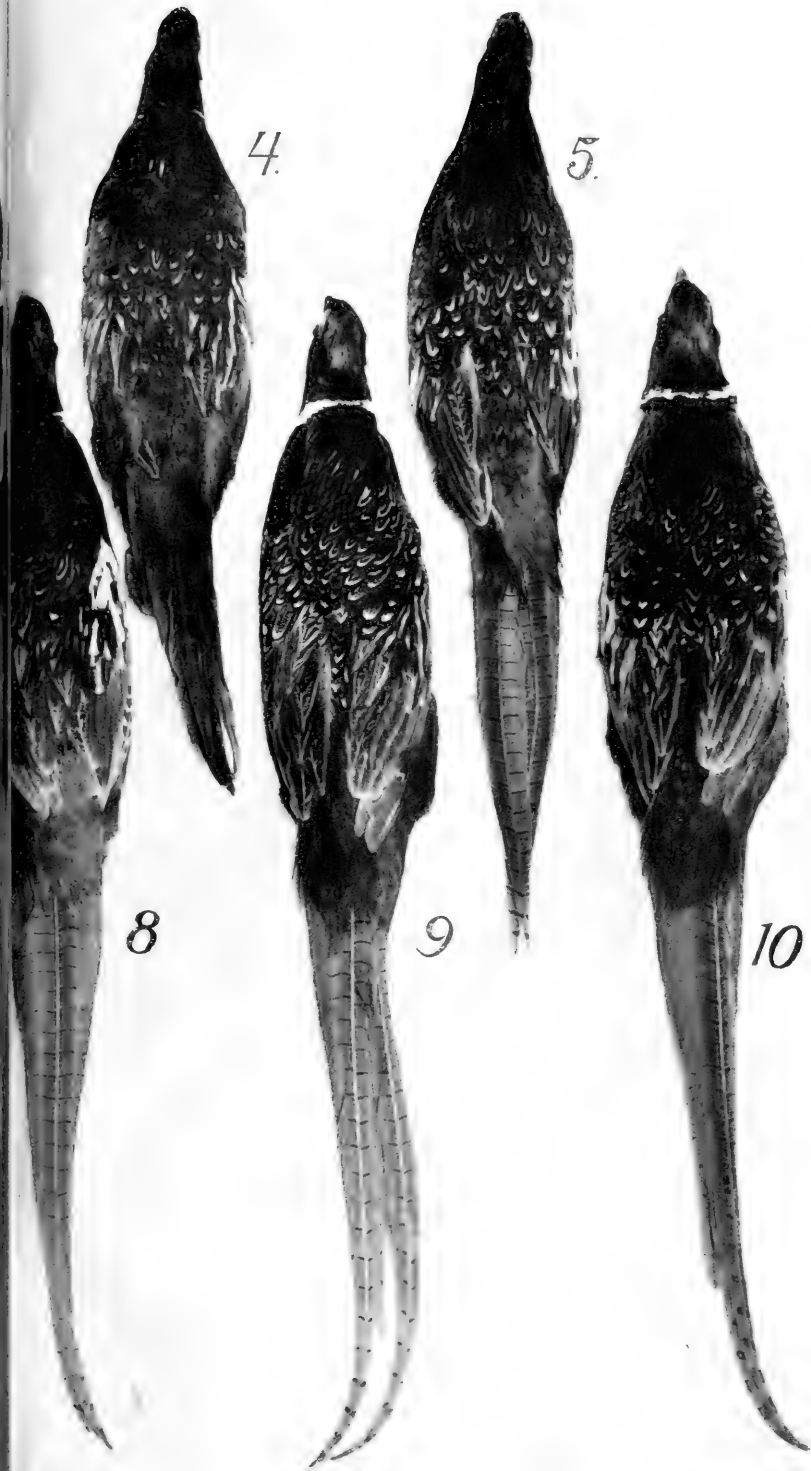


extracted F_2 mallard male practically pure mallard.
extracted F_2 ♀ mallard, showing a bird darker all over than pure mallard,
the lower abdomen sooty: all females are like this.



Prince of Wales \times ring neck experiment.

- 1 Prince of Wales, *P. principalis* ♂.
- 2 Ring neck *P. torquatus*, ♂ (type of ring neck stock).
- 3 F_1 ♂ (intermediate tail bars, rump color, neck-ring and wing coverts).



4, 5 ♂♂ Produced by crossing ♀ F_1 back to *P. principalis* ♂. Figure 6, closest approach to pure *principalis*; No. 831.
 8, 10 F_2 ♂♂ arranged from small neck ring on left to large neck ring on right. Other characters do not show well in plate.



Albino pheasant experiment

- 1 Original spotted ring-neck (torquatus) male dorsal view.
- 2 A typical F_3 generation ♂, from selected F_2 parents; ventral view; some of this series are whiter than this specimen.

PLATE 9

EXPLANATION OF FIGURES

Breast feathers of male ducks

1 Pure black duck *A. tristis* (the fine brownish line probably shows mallard affinities).

2 Pure Australian *A. superciliosa*.

3 Pure mallard, chestnut area, full plumage.

4 Pure mallard, chestnut area, eclipse plumage.

5 Hybrid produced by back crossing F_1 (mallard \times black) with pure mallard; breast feathers of a type intermediate between full mallard plumage and eclipse mallard plumage.

6 F_1 or F_2 . Black \times mallard, showing primitive eclipse mallard feather pattern.

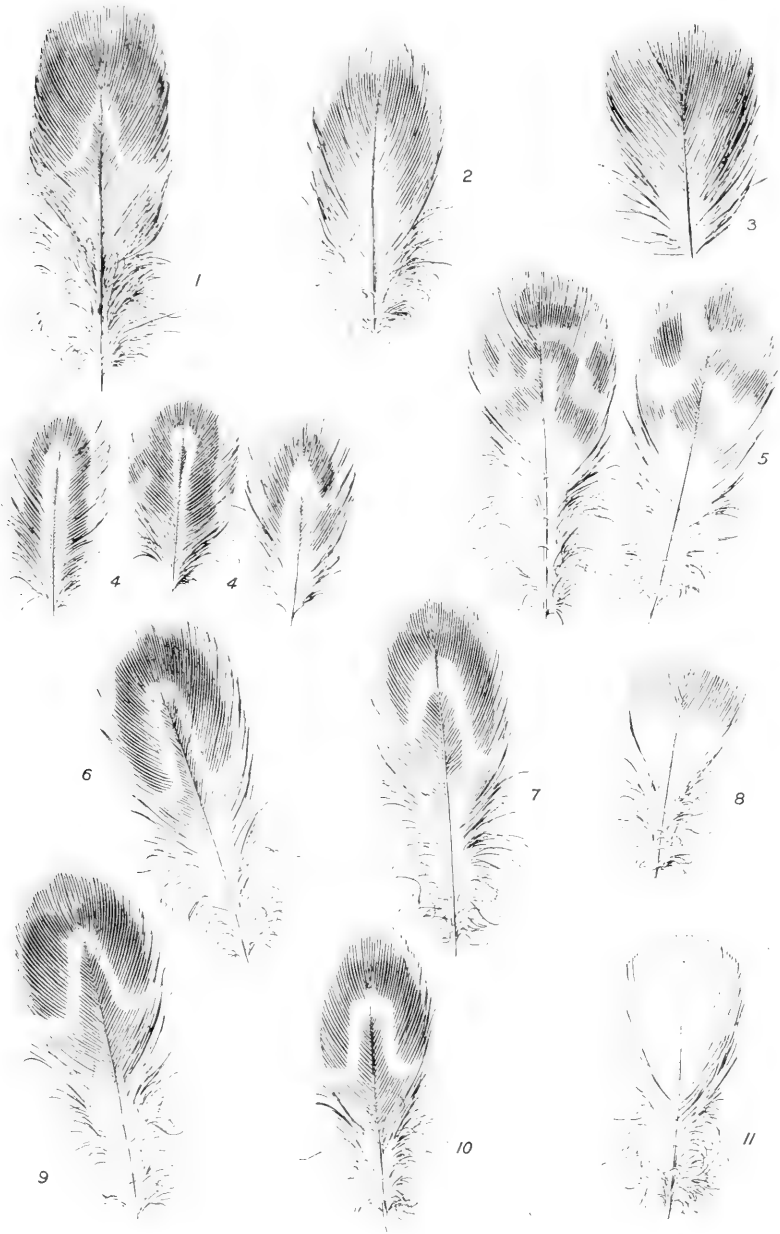
7 F_1 or F_2 . Australian \times mallard, showing primitive eclipse mallard feather pattern.

8 F_1 or F_2 . Pintail \times mallard; shows mallard chestnut, but poorly developed and with no primitive mallard pattern.

9 Three-fourths blood black duck, F_1 (mallard \times black) \times black.

10 Three-fourths blood Australian duck, F_1 (mallard \times Australian) \times Australian. Both figures 9 and 10 preserve the primitive mallard pattern seen in F_1 and F_2 .

11 Three-fourths blood pintail duck F_1 (mallard \times pintail) \times pintail; a faint creamy stain is all that is left of the mallard chestnut.



INHERITANCE IN HYDATINA SENTA

II. CHARACTERS OF THE FEMALES AND THEIR PARTHENOGENETIC EGGS¹

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INTRODUCTION

During the past few years, while working with the rotifer *Hydatina senta*, I have been constantly seeking some feature in regard to which the various parthenogenetic lines obviously differ. Owing to the rapidity of reproduction of this rotifer, and the comparative ease with which matings may be made

¹ Contribution from the Zoölogical Laboratory of the University of Michigan.

whenever males and sexual females are present, such a characteristic would furnish good material for the study of inheritance in what are probably the genetic equivalents of pure lines.

Such a character was first found in the autumn of 1912. At that time I received, through the kindness of Mr. C. F. Rousselet, some fertilized eggs in dried mud scraped from the bottom of a duck pond in England. Almost simultaneously, Prof. J. H. Powers sent some living females collected on the campus of the University of Nebraska. It was at once apparent, when these rotifers were examined, that the eggs of the English line were smaller than those of the Nebraska line. Other characters in which the two lines differed were later discovered. The inheritance of these characters through a number of generations is described in the following pages.

I am much indebted to the gentlemen named above for the material received from them, as well as to Prof. T. H. Morgan for rotifers which were sent at the same time, but which are not involved in this investigation.

DIFFERENTIATING CHARACTERS OF THE TWO LINES

Difference in size of egg

The parthenogenetic eggs of the English line were distinctly smaller than those of the Nebraska line. Although the larger eggs of the former were larger than the smaller eggs of the latter line, each line varied about its own mode, and the difference between the modes was great enough to leave no doubt of its significance. Proof of this will be given presently.

The parthenogenetic eggs of *Hydatina* are approximately ellipsoids. Length and breadth were therefore the measurements required. Measurements were made with a microscope, by means of an ocular micrometer attached to a screw with a graduated head. The screw was used to bring one of the marks of the micrometer tangent to one side or one end of the egg, and the measurement was read at the other side. Fractions of the finest divisions on the micrometer were estimated to tenths. Owing to the personal equation in determining when one of the

lines was tangent to the egg, the estimate of fractions was found to be more accurate (that is, more uniform) than the use of the graduated screw head. All measurements were made with the same microscope and the same combination of lenses. Since only relative sizes are of importance here, the measurements have been left in terms of the divisions of the micrometer. Absolute measurements, if desired, may be obtained, in millimeters, by multiplying the measure here given by 0.0082.

Measurements were usually made without difficulty. The eggs are as a rule cemented to some object, with their long axes parallel to the surface of the object. When they were attached to the bottom of the dish, they were in position to have both length and breadth measured. Practically all measurements were made of eggs on the bottom, though enough were taken at the surface film to make it certain that these did not differ in size from those at the bottom. If an egg did not lie in a horizontal position, that fact should be detected by the difference in focus of the two ends. In case of doubt as to position, the egg was moved with a needle, or measurement was not made.

A necessary precaution regarding measurements of eggs must be mentioned. The late eggs of a female are a little larger than the early eggs, on the average. This is indicated in the summary given in table 1. All the eggs laid by 11 females were measured and recorded in the order in which they were laid. Each family of eggs was then divided into tenths as nearly as possible, and the mean dimensions of the eggs in each tenth computed. As each family comprised 30 to 50 eggs, each tenth included 3 to 5 eggs. In table 1, it is shown that in family 1, for example, the mean dimensions of the eggs of the first tenth of the family were 17.2×14.2 ; in like manner, in family 6, the mean dimensions of the eggs in the eighth tenth were 16.3×14.4 . The mean of these means is then computed for each tenth of the family. For the sake of more ready comparison, a volume coefficient has been calculated as $(\text{Length}) \times (\text{Breadth})^2$. This does not give actual volume, even in units corresponding with the divisions of the micrometer, but it gives a volume coefficient, which is sufficient

TABLE 1

Showing relative size of eggs in different parts of the same family. The unit of measurement is the finest division of an ocular micrometer

FAMILY	MEAN DIMENSIONS OF EGGS IN EACH TENTH OF FAMILY—TENTHS				
	1	2	3	4	5
1	17.2×14.2	16.3×13.8	15.7×13.7	15.9×13.7	16.3×14.1
2	17.6×14.1	17.4×14.6	16.9×14.1	18.2×14.9	18.5×15.0
3	15.6×13.3	16.1×13.2	15.8×13.3	16.1×13.9	16.3×13.8
4	17.1×14.2	17.1×14.1	17.1×14.2	17.6×14.7	17.5×14.4
5	16.4×13.3	15.4×13.5	15.7×13.4	15.6×13.1	16.2×13.0
6	15.8×13.2	15.6×13.5	15.9×13.5	16.1×13.7	16.0×13.6
7	15.3×13.5	15.6×13.4	15.8×13.2	15.9×13.3	16.3×13.5
8	15.4×13.9	15.7×13.1	15.9×13.3	16.2×13.6	16.6×13.5
9	16.0×13.2	15.8×12.9	16.1×13.4	16.4×13.4	16.1×13.8
10	17.2×14.6	17.4×14.7	17.4×14.9	17.6×14.8	17.3×14.8
11	15.8×13.5	15.3×13.6	16.0×13.3	15.8×13.9	16.0×13.2
Mean.....	16.3×13.7	16.2×13.7	16.2×13.7	16.5×13.9	16.6×13.9
Volume coefficient.....	3059	3041	3041	3188	3207

FAMILY	MEAN DIMENSIONS OF EGGS IN EACH TENTH OF FAMILY—TENTHS				
	6	7	8	9	10
1	16.5×14.0	16.4×14.0	16.5×13.9	16.5×14.0	16.8×14.0
2	18.5×15.0	18.4×14.9	18.8×14.8	18.3×15.1	18.5×14.7
3	16.2×13.8	16.2×13.6	17.2×14.3	16.6×14.3	17.1×13.9
4	17.9×14.9	18.1×14.7	18.3×14.5	17.5×14.5	19.5×15.0
5	16.5×13.0	16.3×13.2	17.0×13.0	17.1×14.0	16.8×14.0
6	16.2×14.0	16.2×14.0	16.3×14.4	16.8×14.2	17.4×14.3
7	16.1×13.7	16.2×14.0	16.7×14.2	16.9×14.2	17.1×14.0
8	16.5×13.7	16.9×13.8	16.3×13.8	16.8×13.6	16.9×14.0
9	16.3×13.8	16.5×13.9	16.2×13.7	16.4×14.0	16.9×13.3
10	17.6×15.2	17.1×14.7	17.5×14.9	17.3×14.7	17.6×14.7
11	16.0×13.5	15.6×13.4	16.1×13.4	15.4×13.3	16.0×13.4
Mean.....	16.8×14.1	16.7×14.0	17.0×14.1	16.9×14.2	17.3×14.1
Volume coefficient.....	3340	3273	3380	3408	3439

where only relative volumes are of interest. Actual volume, in cubical units corresponding to the linear units of the micrometer, may be found by multiplying the coefficient of volume by $\frac{\pi}{6}$.

Barring certain fluctuations, there is a gradual increase in the size of the eggs with increasing age of the mother. For this reason, all measurements mentioned below were made upon eggs of the first day's laying in their respective families.

The distribution of the measurements of the eggs of the two original lines is shown in tables 2 and 3. In these tables, in

TABLE 2

Showing the distribution of the eggs of the Nebraska line of Hydatina senta, with respect to their length and breadth. The unit of measurement is the finest division of an ocular micrometer

NUMBER OF EGGS OF BREADTHS GIVEN BELOW	NUMBER OF EGGS OF LENGTHS GIVEN BELOW								
	16.0	16.5	17.0	17.5	18.0	18.5	19.0	19.5	20.0
13.5	1	2					1		
14.0	2	1	12	5	8	8	1		
14.5		3	12	13	10	11	5		1
15.0	1		8	13	24	7	14	3	
15.5				2	6	1	4		
16.0					3	2		1	

Number of eggs measured, 185; mean length, 17.90 ± 0.058 ; mean breadth, 14.69 ± 0.043 ; $\sigma_L = 0.786 \pm 0.041$; $\sigma_B = 0.590 \pm 0.030$.

TABLE 3

Showing the distribution of the eggs of the English line of Hydatina senta, with respect to their length and breadth. The unit of measurement is the finest division of an ocular micrometer

NUMBER OF EGGS OF BREADTHS GIVEN BELOW	NUMBER OF EGGS OF LENGTHS GIVEN BELOW							
	14.5	15.0	15.5	16.0	16.5	17.0	17.5	18.0
13.0		2	4	13	8	3	1	
13.5		3	8	17	10	2		
14.0	3	1	7	18	16	28	6	3
14.5			2	4	6	5	4	4
15.0					1	2		1

Number of eggs measured, 182; mean length, 16.40 ± 0.060 ; mean breadth, 13.81 ± 0.039 ; $\sigma_L = 0.804 \pm 0.042$; $\sigma_B = 0.522 \pm 0.028$.

order to increase the number of eggs in each size class, the class interval is taken as one-half the finest division of the ocular micrometer. All eggs recorded in my notes as measuring 16.3 to 16.7, inclusive, appear in these tables as measuring 16.5; all first recorded 16.8 to 17.2, inclusive, are here given as 17.

The mean dimensions of the English eggs are $(16.40 \pm 0.060) \times (13.81 \pm 0.039)$. Those of the Nebraska line are $(17.90 \pm 0.058) \times (14.69 \pm 0.043)$. The difference between the mean lengths is 1.50 ± 0.083 ; the difference between the mean breadths is 0.88 ± 0.058 . The difference is 18 times its mean error in the former case, 15 times its error in the latter; hence there can be no doubt that the differences are significant.

Difference in time of egg development

When two lots of eggs laid at the same time, one by the English line, the other by the Nebraska line, were reared to hatching, it was noted that those of the Nebraska line invariably began to hatch first. The time of development was more accurately determined for each line in the following manner. A number of females were placed together in a dish, and allowed to remain twenty minutes to an hour and a half. At the end of that time the females were removed. The eggs laid in the dish were preserved, and after a period of about twelve hours were examined every half hour, or thereabouts. As the young females hatched, they were removed and counted. Record was kept of the time in which the eggs were laid, and the time at which each lot of young females was later removed. It is assumed that the eggs were laid uniformly throughout the period of laying, and that, for the purpose of computing the mean time of development, the middle of the laying period may be taken as the time of laying of all the eggs. Likewise, the females removed at each later examination were reckoned as having hatched at the middle of the period elapsing since the last preceding examination. This method of determining the time of egg development introduces some errors, but when large numbers of eggs were used the errors could not have affected the mean to any great extent. It does

affect the variability of the time of development, as is explained below.

Records of the time of development were kept at four different times during the whole investigation. The mean time of development, as might be expected, is not the same in all these periods. The four sets of records are therefore kept separate. Tables 4 and 5 show the results obtained. The time of development is given in table 4 to the nearest hour.

In July, the mean time of development of the eggs of the English line was 14.98 ± 0.21 hours; of the Nebraska line, 13.33 ± 0.08 hours. The difference between the two means, as shown in the

TABLE 4

Showing the distribution of the eggs of the English and Nebraska lines of Hydatina senta, with respect to the time required for development

LINE	DATE	TIME OF EGG DEVELOPMENT, IN HOURS											
		12	13	14	15	16	17	18	19	20	21	22	23
Eng.	July.....		21	23	15	10	6	4	2	3	1		
	Nov.....			3	6	9	16	8	5	0	4		
	Dec.....			1	3	7	6	8	4	2	3	1	
	Mar.-Apr....				2	6	4	10	11	9	4	1	1
Neb.	July.....	18	75	21	19	1							
	Nov.....		2	10	28	14							
	Dec.....		1	16	38	19	9						
	Mar.-Apr....			1	20	18	15	8					

TABLE 5

Showing mean time of development, and variability of time of development, of the eggs of the English and Nebraska lines of Hydatina senta, as computed from table 4

LINE	DATE	NUMBER OF EGGS	MEAN TIME OF DEVELOPMENT, IN HOURS	STANDARD DEVIATION OF TIME OF DEVELOPMENT	EXCESS OF MEAN OVER THAT OF ENGLISH LINE	EXCESS OF STANDARD DEVIATION OVER THAT OF ENGLISH LINE
Eng.	July.....	85	14.98 ± 0.21	1.94 ± 0.15		
	Nov.....	51	17.08 ± 0.24	1.72 ± 0.17		
	Dec.....	35	17.66 ± 0.33	1.94 ± 0.23		
	Mar.-Apr....	44	18.55 ± 0.28	1.87 ± 0.20		
Neb.	July.....	134	13.33 ± 0.08	0.90 ± 0.06	-1.65 ± 0.22	-1.04 ± 0.16
	Nov.....	54	15.00 ± 0.10	0.77 ± 0.07	-2.08 ± 0.26	-0.95 ± 0.18
	Dec.....	83	15.23 ± 0.10	0.92 ± 0.07	-2.43 ± 0.34	-1.02 ± 0.24
	Mar.-Apr....	62	16.15 ± 0.14	1.06 ± 0.10	-2.40 ± 0.31	-0.81 ± 0.22

sixth column of table 5, is nearly eight times its mean error, hence there can be no doubt of its significance. In November, the time of development was greater for both lines, but again that of the English line was greater than that of the Nebraska eggs. December and March to April gave similar results. The reasons for the greater time required for development in the later determinations have not been fully determined, though temperature is a potent factor.

Another striking fact shown in tables 4 and 5 is that the variability of the time of development is greater in the English line than in the Nebraska line. This was a matter of frequent observation throughout the experiments. A lot of Nebraska eggs laid in a period of twenty minutes were practically all hatched within a period of an hour; English eggs, on the other hand, if laid in a period of twenty minutes, hatched irregularly over a period of four or five hours. The contrast between the two degrees of variability was much more striking than the standard deviation here given would indicate. There are two reasons for this. First, when the eggs of a given lot were laid during a period of an hour and a half, the period of hatching was necessarily greater than when the eggs were laid in twenty minutes. As this erroneous increase was as great for the Nebraska line as for the English line, the ratio of the standard deviation of the latter to that of the former was diminished (because $\frac{y+m}{x+m}$ is less than $\frac{y}{x}$, where y is greater than x and m is a positive quantity). Second, within the month of July, for example, the time of development was not the same on all days. In order that the results might be handled statistically, all these daily records have been combined. There is thus created an appearance of variability that would be diminished if all the determinations had been made on one day, or under one set of conditions.

The difference between the standard deviations of the time of development of the two lines is, according to table 5, four to six times its mean error. Since, for the two reasons given above, both standard deviations are too high, and their mean errors are

therefore also too high, the difference between the two standard deviations should exceed its own mean error more than table 5 indicates. It seems certain, therefore, that the difference is significant.

Difference in rate of egg production

The rate of egg production was found at once to be unequal in the two lines, when they were reared side by side under the same conditions. Every day more young females were isolated from the dishes containing the English females than from those of the Nebraska line. The difference in the rate of egg laying was determined as follows. A given small number of females, all of which were known to have begun laying, were put into a

TABLE 6

Showing rate of egg laying of the English and Nebraska lines of Hydatina senta

LINE	NUMBER OF FEMALES LAYING	NUMBER OF DAYS LAYING	NUMBER OF INDIVIDUAL DAYS OF LAYING	NUMBER OF EGGS LAID	NUMBER OF EGGS PER DAY PER FEMALE	VOLUME COEFFICIENT OF EGG OF MEAN DIMENSIONS	VOLUME COEFFICIENT OF TOTAL EGGS OF ONE FEMALE IN ONE DAY
English.....	14	1.99	27.86	418	15.0	3,123	46,845
Nebraska.....	22	1.57	34.54	420	12.2	3,868	47,189

dish, and removed a day or two later. The number of eggs laid in that time was determined by counting the females that hatched from them later. If, as happened several times, any of the adult females whose egg laying was being tested, died before removal, or showed by their condition that they had not been laying eggs recently, the dish containing them was rejected. The dishes usually contained only two or three females, in order that the conditions might remain at the optimum for a longer period. The results are summarized in table 6.

In table 6, the number in column four is the product of those in columns two and three. The number in the sixth column is obtained by dividing the number of eggs in the fifth column by the number of days in the fourth column. The volume coefficient in the seventh column is computed from the mean dimen-

sions of the eggs of the respective lines, as given in tables 2 and 3, and is calculated in the manner described for table 1. The number in the last column is the product of the numbers in the two next preceding columns. Other determinations of the rate of egg production in the same lines were made at a later date, and used as controls, as shown in table 13; but because no eggs of these lines were then being measured, the counts are not included in table 6, where egg volumes are concerned.

From the last column of table 6 it appears that the aggregate volume of egg substance produced in a day by an English female was nearly as much as that produced by a Nebraska female. The smaller size of the English eggs is not due, therefore, to a slower rate of metabolism involved in egg production, but to a tendency to put up the same quantity of substance in smaller packages.

Difference in place of egg laying

While measurements of eggs were being made, it was noted that almost invariably most of the Nebraska eggs were cemented to the bottom of the dish, whereas a large proportion of the English eggs were held at the surface film of the water. Measurement of this feature of the lines was made as follows. Into each of two dishes, and in equal quantities of water, were put approximately equal numbers of females of the two lines, respectively. They were left in the dishes during the same period of time. An effort was made to provide food that would not float at the surface film nor adhere to it, so that eggs might be laid elsewhere.

Table 7 shows the number of eggs found at the bottom of these dishes, and at the surface of the water. Counts were made in both lines on the same days, at intervals from July to December, 1913, and again in March and June, 1914, partly on the same days.

The determinations made from July to December show a much higher percentage of eggs at the surface in the English line than in the Nebraska line. I know of no satisfactory way of handling statistically the records of the location of eggs, in order to prove that the difference between the lines is significant. Had all the counts been made on one day, or under one set of

conditions, statistical treatment would be simple. Simultaneous tests were made in both lines, as shown in table 7, on 16 different days. If, as seems practically certain, the location of the eggs is affected by external conditions, there should be, from day to day, a fluctuation of the percentage of the eggs at the surface. Such a fluctuation does, in fact, appear in the records. The count for each day might serve, therefore, as a unit. But 16 units are too few for statistical treatment; and there is the further objection that, though the percentage of eggs fluctuated, it usually shifted in the same direction in both lines. When an unusually large number of eggs were at the surface in the English line, the Nebraska line also showed in most cases more than the

TABLE 7

Showing number of eggs laid on bottom of dish and at surface film of water, by the English and Nebraska lines of Hydatina senta

DATE	ENGLISH LINE			NEBRASKA LINE		
	Number of eggs at surface	Number of eggs at bottom	Per cent at surface	Number of eggs at surface	Number of eggs at bottom	Per cent at surface
July 19, 1913.....	29	8	54.1	15	34	9.9
20	60	135		0	142	
21	48	0		12	33	
22	68	10		10	47	
23	53	64		9	108	
25	154	66		22	110	
27	68	3		21	66	
28	26	82		0	42	
29	97	0		18	71	
30	15	74		0	89	
Aug. 1	99	88	88.9	6	75	21.4
2	67	81		0	76	
Oct. 5	10	28		0	27	
Nov. 16	21	34		3	67	
Dec. 8	16	33		2	80	
Mar. 16, 1914.....				23	74	
				30	62	
				15	82	
June 9	84	6		32	146	
	86	9		20	75	
	132	21				
12	68	10				

average number at the surface (see July 19, 21, 22, 25, 27, and 29). Fewer than the average number at the surface also appeared in both lines on the same days (see July 20, 28, 30, August 2, October 5, November 16, and December 8). If this correspondence in the fluctuation of the two lines were ignored, and each day's count used as a unit for statistical treatment, there might be thereby created an appearance of fluctuation so great that the mean error would cast doubt upon the significance of the difference of the means of the two lines. In view of the fact that both lines usually shifted in the same direction, and every day showed a difference of the same sign, such a doubt can scarcely be entertained.

What physiological factors cause the eggs of one line to be laid at the surface, those of the other at the bottom, have not been determined. It may be that the fundamental cause is the demand for oxygen. I owe to Dr. O. C. Glaser the suggestion that the difference in the location of the eggs may be due to a difference in the permeability of the body of the females. Some brief experiments to test this supposition do not support the view, though the tests can not be regarded as conclusive. Healthy females of both lines, all of about the same age, were placed simultaneously in a $\frac{N}{20}$ solution of lithium chloride, and examined at intervals of one minute until all muscular movement ceased. Ciliary movement continued longer, but as it was hard to decide when it stopped, that movement was ignored. On different days, the time required to kill the rotifers varied from 19 to 26 minutes; but on the same day, the time required to stop muscular activity was almost exactly the same, to the minute, in both lines. Only about a dozen females of each line were tested with lithium chloride. A few similar tests with 0.5 per cent solution of sodium chloride gave essentially the same results, but the time was longer. Further discussion of permeability as affecting other characters than the location of the eggs is given beyond.

Some brief preliminary experiments indicate that, of external agents, temperature modifies the proportion of the eggs laid at the surface.

Difference in contractility of the foot muscles

A series of muscle bands extends from the sides of the body to the foot, enabling the animal to telescope the foot more or less completely within the rest of the body. When a number of females were killed in Bouin's fluid, it was found that the foot of the Nebraska females was retracted, on the average, much more fully than that of the English females. I thought at first that this might be due to the mechanical effect of a difference in the size of the body. The Nebraska females were noticeably larger than the English rotifers, a difference which can not well be measured in the living females, and which is not therefore discussed under a separate caption. Since, before killing the rotifers in Bouin's fluid, as much water as possible was first drawn off, it seemed possible that the larger bodies of the Nebraska females were caught more firmly between the surface film of water and the bottom of the dish, and that this mechanical stimulus resulted in the greater contraction of the muscles of the one line. This was shown not to be the case, however, by comparing young Nebraska females with old English females, the latter being the larger. The Nebraska females again showed the greater degree of foot retraction. Further proof that the retraction was not due to the mechanical stimulus of the film of water was found in the behavior of the rotifers when subjected to various salts. The rotifers were, in these tests, in an abundance of water, hence there was no disturbance by the surface film. The Nebraska females responded to all these chemical stimuli, if of the proper strength, by first retracting the foot wholly within the body, and resting for a time on the bottom of the dish. After they resumed swimming, they squirmed at frequent intervals and partly or wholly retracted the foot for a short time. The English rotifers, on the other hand, while performing moderate squirming movements, did not usually retract the foot within the body. It seems necessary to assume, therefore, that the two lines differed in the degree to which their foot muscles responded to stimuli.

To measure this quantitative character, in the absence of any natural units of measurement, the following classes or degrees of contraction were arbitrarily established. 1. Fully extended, sides of foot smooth. 2. Slightly contracted, sides of foot somewhat wrinkled. 3. Moderately contracted, but with toes wholly protruding from the body. 4. Greatly contracted, toes partly or wholly concealed within the body. All tests recorded were made by killing the animals in Bouin's fluid, because specimens were being preserved for cytological study.

TABLE 8

Showing distribution of the females of the English and Nebraska lines of Hydatina senta, with respect to the degree of contraction of the foot muscles (see text for further description of class numbers)

LINE	NUMBER OF FEMALES SHOWING FOLLOWING DEGREES OF CONTRACTION				TOTAL NUM- BER OF ♀ ♀	MEAN CLASS NUMBER	STANDARD DEVI- ATION	EXCESS OF MEAN OVER THAT OF ENGLISH LINE
	1 Fully ex- tended	2 Slightly contracted	3 Moderately contracted	4 Greatly contracted				
Eng.....	34	13	3	1	51	1.39 ± 0.101	0.72 ± 0.071	
Neb.....	1	5	25	81	112	3.66 ± 0.057	0.61 ± 0.040	$+2.27 \pm 0.116$

Table 8 shows the number of these fixed specimens falling within each of the above four classes. The mean class number of the English line is 1.39 ± 0.101 , that of the Nebraska line, 3.66 ± 0.057 . The difference between these means, as shown in the last column of table 8, is nearly 20 times its mean error, which leaves little doubt of the significance of the difference.

INHERITANCE OF DIFFERENTIATING CHARACTERS

Reciprocal crosses between the English and Nebraska lines were effected with some difficulty. Mating was not infrequently observed, but the number of sexual females, and hence also of males, was quite small in both lines, so that the simultaneous occurrence of males in one line and sexual females in the other was not common. Furthermore, many matings were failures; the female of a pair often proved to be sexual, but her eggs had

not been fertilized. The difficulty with which fertilization was brought about was new to me, and may indicate some degree of incompatibility between the lines.

A more serious obstacle to crossing was the fact that only a small percentage of the fertilized eggs hatched. The proportion of viable eggs is, as I have shown elsewhere (Shull '13), a heritable character, and varies in different lines. In the lines here described and in all their progeny, the proportion of viable eggs was small. Many ways have been tried to cause the eggs to hatch, but so far without success. Hatching may be prevented, and it may be hastened, but no way of inducing it has been discovered.

Notwithstanding these difficulties, enough results have been obtained to be of some theoretical interest. Four successive generations in the direct line have been obtained, and two generations from a back cross. No viable eggs were obtained from the last of these generations. Table 9 presents in concise form the relationship of all the lines studied. All lines except the original parent lines are designated by numbers. It is seen that in F_1 six viable lines were obtained, out of about 60 fertilized eggs. Fortunately, these six lines include representatives of both reciprocal crosses. In F_2 , six viable lines were secured from about 48 fertilized eggs. In F_3 , 15 lines from about 80 fertilized eggs, and so on. By F_3 back cross I mean the progeny obtained by crossing an F_2 with one of its grandparental lines, as is explained in the table. For convenience, the inheritance of all the differentiating characters of the original lines is shown in tabular form, in the same order as in table 9.

Each differentiating trait will now be taken up in turn, and its inheritance through the various lines presented.

Inheritance of size of egg

Table 10 gives, in summary form, the results of the measurements of the eggs in all the lines studied. By including in this table the standard deviation of both length and breadth, it is believed that the distribution of the eggs of each line, with regard to their dimensions, is sufficiently described. It is not necessary, therefore, to tabulate each line separately, as was done for the

English and Nebraska lines. With the aid of table 9, the relationship of each line to all the rest may be easily discovered, and the significance of the results here presented readily understood. Lines 95 to 99, inclusive, died out because of unfavorable conditions before their eggs were measured. Line 48, from the fertilized inbred eggs of the Nebraska line, comprised only one adult female, which laid five eggs. None of the females hatching from these eggs reached maturity. The mean dimensions of the five eggs were 17.9×14.2 , or almost as large as the Nebraska eggs. In line 104, only seven eggs were measured, the mean dimensions of these being 18.8×15.7 . In the remaining lines an adequate number of eggs was measured.

Attention should be directed to the following points, most readily shown in the last two columns of table 10. Lines 80 and 81, from inbred eggs of the English line, laid eggs that did not differ materially from those of the English line; certainly they were not any larger. The three lines derived from crosses between the English and Nebraska lines (44, 47, and 49) were all practically the same, in egg size, as the English line, as if small egg size were dominant. These three lines as shown in table 9, represent both reciprocal crosses.

All F_2 lines laid eggs at least as small as the English eggs. There is no evidence of inheritance from the large-egged Nebraska line. There is no appearance of segregation, with respect to egg size, in F_2 .

In all F_3 lines except line 61, the eggs are of practically the same size as the English eggs. Line 61 laid eggs somewhat longer. The difference between line 61 and the English line is more than eight times its mean error, and is probably significant, especially in view of the results in F_4 . In F_3 , then, the only evidence of inheritance of size of egg from the Nebraska line is in line 61.

In F_4 , the lines are probably not significantly different from the English line, except line 75, and perhaps line 79. In the former, the excess of the length of the eggs over that of the English eggs was nearly six times its mean error; in the latter the difference in breadth was five times its mean error. I believe these results

TABLE 9

Showing origin and relationship of all parthenogenetic lines of Hydatina senta whose inheritance is described in the text. All lines except the first parental lines are designated by numbers

GENERA- TION	NUM- BER OF FER- TILIZED EGGS	NUMBERS OF LINES	ORIGIN OF LINES
P ₁		English Nebraska	
F ₁	60	48..... 80, 81..... 44..... 47, 49.....	From fertilized egg of inbred ♀ of Neb. line From fertilized eggs of inbred ♀ ♀ of Eng. line From fertilized egg of ♀ of Neb. line, crossed with ♂ of Eng. line From fertilized eggs of ♀ ♀ of Eng. line crossed with ♂ ♂ of Neb. line
F ₂	48	50..... 51, 54..... 55, 56, 57.....	From fertilized egg of inbred ♀ of line 49 From fertilized eggs of inbred ♀ ♀ of line 44 From fertilized eggs of inbred ♀ ♀ of line 47
F ₃	80	58, 59, 60, 61, 62, 63, 66, 67, 68, 69, 70, 71, 72... 64..... 65.....	From fertilized eggs of inbred ♀ ♀ of line 56 From fertilized egg of inbred ♀ of line 57 From fertilized egg of inbred ♀ of line 50
F ₄	45	73..... 74..... 75, 76, 77, 78, 79.....	From fertilized egg of inbred ♀ of line 64 From fertilized egg of inbred ♀ of line 59 From fertilized eggs of inbred ♀ ♀ of line 61
F ₃ back- cross	56	82, 83, 84, 85, 86.....	From fertilized eggs of ♀ ♀ of line 56, crossed with ♂ ♂ of Neb. line
F ₄ from back- cross	114	87, 88, 89, 108.. 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 102, 103, 104, 105, 106, 107.. 101.....	From fertilized eggs of inbred ♀ ♀ of line 84 From fertilized eggs of inbred ♀ ♀ of line 85 From fertilized egg of inbred ♀ of line 82

TABLE 10

Showing size of egg and variability of size of egg, of the lines of Hydatina senta described in this paper

GENERATION	LINE	NO. OF EGGS MEASURED	MEAN LENGTH	MEAN BREADTH	STANDARD DEVIATION OF LENGTH	STANDARD DEVIATION OF BREADTH	EXCESS OF MEAN LENGTH OVER THAT OF ENGLISH LINE	EXCESS OF MEAN BREADTH OVER THAT OF ENGLISH LINE
P ₁	Eng.	182	16.40±0.060	13.81±0.039	0.80±0.042	0.52±0.028		
	Neb.	185	17.90±0.058	14.69±0.043	0.79±0.041	0.59±0.030	+1.50±0.083	+0.88±0.058
F ₁	48	5	(see text)					
	80	57	16.09±0.062	13.62±0.042	0.47±0.044	0.32±0.030	-0.31±0.086	-0.19±0.057
	81	37	16.19±0.092	13.58±0.060	0.56±0.065	0.37±0.042	-0.21±0.109	-0.23±0.071
	44	140	16.41±0.060	13.84±0.040	0.71±0.042	0.48±0.028	+0.01±0.084	+0.03±0.056
	47	144	16.36±0.052	13.87±0.037	0.62±0.037	0.45±0.026	-0.04±0.079	+0.06±0.054
	49	38	16.20±0.154	13.71±0.092	0.95±0.109	0.57±0.065	-0.20±0.165	-0.10±0.100
F ₂	50	258	16.28±0.047	13.62±0.030	0.75±0.033	0.49±0.021	-0.12±0.076	-0.19±0.049
	51	64	16.11±0.070	13.56±0.052	0.56±0.049	0.41±0.037	-0.29±0.092	-0.25±0.065
	54	147	15.96±0.050	13.50±0.034	0.61±0.035	0.41±0.024	-0.44±0.078	-0.31±0.052
	55	65	16.15±0.069	13.63±0.056	0.56±0.049	0.45±0.040	-0.25±0.091	-0.18±0.069
	56	59	16.36±0.079	13.76±0.065	0.61±0.056	0.50±0.046	-0.04±0.099	-0.05±0.076
	57	72	16.49±0.077	13.74±0.053	0.65±0.054	0.45±0.037	+0.09±0.097	-0.08±0.066
F ₃	58	93	16.51±0.077	13.88±0.058	0.74±0.054	0.56±0.041	+0.11±0.097	+0.07±0.070
	59	75	16.44±0.080	13.74±0.067	0.69±0.057	0.58±0.047	+0.04±0.100	-0.07±0.077
	60	77	16.41±0.070	13.77±0.052	0.61±0.049	0.46±0.036	+0.01±0.092	-0.04±0.065
	61	114	17.14±0.067	13.85±0.049	0.71±0.047	0.52±0.034	+0.74±0.090	+0.04±0.063
	62	110	16.37±0.066	13.75±0.048	0.70±0.047	0.50±0.034	-0.03±0.089	-0.06±0.061
	63	91	16.38±0.078	13.68±0.049	0.74±0.055	0.47±0.035	-0.02±0.098	-0.13±0.063
	66	127	16.38±0.059	13.70±0.042	0.66±0.042	0.48±0.030	-0.02±0.084	-0.11±0.057
	67	106	16.33±0.065	13.76±0.044	0.67±0.046	0.45±0.031	-0.07±0.088	-0.05±0.059
	68	55	16.53±0.106	13.59±0.060	0.78±0.075	0.44±0.042	+0.13±0.122	-0.22±0.071
	69	68	16.47±0.079	13.71±0.054	0.65±0.056	0.45±0.038	+0.07±0.099	-0.10±0.067
	70	82	16.45±0.083	13.65±0.052	0.75±0.059	0.47±0.037	+0.05±0.102	-0.16±0.065
	71	93	16.40±0.085	13.65±0.046	0.82±0.060	0.45±0.033	0.00±0.104	-0.16±0.066
	72	90	16.26±0.077	13.69±0.043	0.73±0.054	0.40±0.030	-0.14±0.097	-0.12±0.058
	64	99	16.38±0.072	13.70±0.059	0.72±0.051	0.58±0.042	-0.02±0.094	-0.11±0.071
	65	99	16.18±0.079	13.83±0.046	0.78±0.056	0.46±0.033	-0.22±0.099	+0.02±0.060
F ₄	73	67	16.36±0.083	13.74±0.048	0.68±0.059	0.40±0.034	-0.01±0.102	-0.07±0.061
	74	91	16.62±0.086	13.87±0.046	0.82±0.061	0.44±0.033	+0.22±0.104	+0.06±0.060
	75	100	17.01±0.085	13.95±0.044	0.85±0.060	0.44±0.031	+0.61±0.104	+0.14±0.059
	76	118	16.38±0.065	14.07±0.046	0.71±0.046	0.50±0.033	-0.02±0.088	+0.26±0.060
	77	136	16.18±0.058	13.79±0.038	0.69±0.041	0.45±0.027	-0.22±0.083	-0.02±0.054
	78	60	16.52±0.090	13.87±0.052	0.70±0.064	0.40±0.037	+0.12±0.108	+0.06±0.065
	79	70	16.86±0.118	14.23±0.076	0.99±0.083	0.64±0.054	+0.46±0.132	+0.42±0.085
F ₅ back-cross	82	57	16.51±0.080	14.04±0.043	0.60±0.057	0.32±0.030	+0.11±0.100	+0.23±0.058
	83	59	15.95±0.059	13.81±0.062	0.45±0.042	0.48±0.044	-0.45±0.084	0.00±0.073
	84	59	16.00±0.063	13.87±0.048	0.49±0.045	0.37±0.034	-0.40±0.087	+0.06±0.061
	85	66	16.32±0.068	14.03±0.039	0.55±0.048	0.31±0.028	-0.08±0.091	+0.22±0.055
	86	62	16.43±0.059	13.92±0.039	0.46±0.042	0.31±0.028	+0.03±0.084	+0.11±0.055
F ₄ from back-cross	87	81	16.29±0.054	13.68±0.038	0.49±0.038	0.35±0.027	-0.11±0.081	-0.13±0.054
	88	81	16.27±0.054	13.51±0.040	0.49±0.038	0.36±0.028	-0.13±0.081	-0.30±0.056
	89	67	16.29±0.054	13.71±0.046	0.44±0.038	0.38±0.033	-0.11±0.081	-0.10±0.060
	108	64	16.80±0.070	13.76±0.045	0.56±0.049	0.36±0.032	+0.40±0.092	-0.05±0.059

TABLE 10—Continued

GENERATION	LINE	NO. OF EGGS MEASURED	MEAN LENGTH	MEAN BREADTH	STANDARD DEVIATION OF LENGTH	STANDARD DEVIATION OF BREADTH	EXCESS OF MEAN LENGTH OVER THAT OF ENGLISH LINE	EXCESS OF MEAN BREADTH OVER THAT OF ENGLISH LINE
F ₄ from back-cross	90	64	18.23±0.096	14.68±0.069	0.77±0.068	0.55±0.049	+1.83±0.113	+0.87±0.079
	91	70	16.36±0.060	13.61±0.045	0.50±0.042	0.38±0.032	-0.04±0.084	-0.20±0.059
	92	49	16.27±0.076	13.51±0.047	0.53±0.054	0.33±0.033	-0.13±0.097	-0.30±0.061
	93	49	18.61±0.131	14.91±0.076	0.92±0.093	0.53±0.054	+2.21±0.144	+1.10±0.085
	94	49	18.51±0.084	14.79±0.061	0.59±0.059	0.43±0.043	+2.11±0.103	+0.98±0.072
	100	64	16.80±0.071	14.03±0.029	0.57±0.050	0.23±0.021	+0.40±0.093	+0.22±0.048
	102	36	16.24±0.087	13.67±0.075	0.52±0.062	0.45±0.053	-0.16±0.105	-0.14±0.084
	103	49	17.98±0.123	14.61±0.099	0.86±0.087	0.69±0.070	+1.58±0.136	+0.80±0.106
	104	7	(see text)					
	105	36	16.33±0.098	13.81±0.077	0.59±0.069	0.46±0.054	-0.07±0.115	0.00±0.086
	106	36	18.35±0.138	14.85±0.088	0.83±0.098	0.53±0.062	+1.95±0.150	+1.04±0.096
	107	36	18.08±0.140	14.67±0.087	0.84±0.099	0.52±0.062	+1.68±0.152	+0.86±0.095
	101	36	16.26±0.123	13.83±0.087	0.74±0.087	0.52±0.062	-0.14±0.136	+0.02±0.095

to be significant. Both of these lines, as shown in table 9, were derived from line 61, which itself laid eggs larger than the English eggs.

An F₅ generation could not be obtained to test further the inheritance of egg size in lines 75 and 79, for the few fertilized eggs that were laid in these lines did not hatch. It is believed, however, that the larger eggs of line 61, and its daughter lines 75 and 79, are the effects of inheritance from the Nebraska parent line. For further tests of this inheritance, recourse was had at this point to a back cross between an F₂ line and the Nebraska line. Line 56 was chosen from F₂ because it was producing many sexual females, so that crossing was not difficult.

In the back crosses, the eggs of no line are significantly larger than those of the English greatgrandparent. The appearance is again that of simple dominance of small egg size.

In the F₄ series of lines derived from the back cross, however, several possessed eggs unmistakably larger than the English eggs. The eggs of lines 103 and 107 were as large as Nebraska eggs, while those of lines 90, 93, 94, and 106 were even larger. In lines 108 and 100, the mean length is somewhat greater than that of the English eggs, though the difference is of very doubtful significance. If line 108 be not included, all the lines producing large eggs were derived from line 85, whose eggs were not significantly larger than those of the English line.

To summarize, all F_1 lines, all F_2 lines, 14 out of 15 F_3 lines, at least 5 out of 7 F_4 lines, all the back cross lines, and at least 8 out of 16 F_4 lines from the back cross, laid eggs as small as the English line. Of the remaining lines, the one in F_3 , and one in F_4 were intermediate between English and Nebraska; one in F_4 and two in F_4 from the back cross laid eggs that were not certainly significantly larger than English eggs; while two in F_4 from the back cross laid eggs as large as Nebraska eggs, and four in F_4 from the back cross laid eggs even larger than the Nebraska eggs.

Large size of eggs appears much less frequently than would be expected if egg size were a simple Mendelian character, especially in the first three filial generations.

Inheritance of time of egg development

Tables 11 and 12 give the results of the determinations of the time of development of the eggs of all lines described in this paper, except lines 48 and 49. These two lines were no longer in existence when work on the time of development began. Table 11 gives the number of hours required for development, table 12 the means, standard deviations, differences of means, etc. The last two columns of table 12 show the significance of the results. The English and Nebraska lines are repeated from tables 4 and 5 for comparison. All lines are given, as before, in the same order as in table 9.

The following points are worthy of attention. With respect to mean time of development, no line in F_1 , F_2 , or F_3 , differs significantly from the English line. In F_4 , the difference of line 76 may be significant, but is of the wrong sign to be attributed to inheritance from the Nebraska line. The F_3 back cross includes no line approaching significantly the time of development of the Nebraska eggs, while in F_4 from this back cross, only the eggs of line 94 appear to have developed in significantly less time than the English eggs. The time of egg development in line 94 is intermediate between the time of development of the English eggs and the Nebraska eggs.

To summarize, in all the experiments here recorded, only one line (94) shows any indication of an inheritance of the short time of development of the Nebraska line.

With respect to the variability of the time of development, the results are less definite. The Nebraska line showed much less variability than the English line, as was shown in tables 4 and 5, and the difference was shown to be even greater than those tables indicated. In the filial generations most lines showed less variability than the English line, as is indicated by the preponderance of *minus* signs in the last column of table 12. But the mean error of the standard deviation is so high that the difference is always of doubtful significance. In line 54 in F_2 and line 103 in F_4 from the back cross, the differences are greatest, and are here perhaps significant.

Inheritance of rate of egg production

The possible inheritance of the rate of egg production was tested through three filial generations, and in one line of F_4 . In December, 1913, it became necessary either to abandon some part of the work undertaken or to do all of it less completely. As the results of the study of the inheritance of the rate of egg production up to that time were less definite than any other results, and as enormous fluctuations in the rate of egg production by the same individual at different times, and by different individuals of one line at the same time, required that large numbers of records be obtained in order to make the results trustworthy, this part of the investigations was dropped. The records, as far as obtained, are given for what they are worth, in table 13.

The F_2 lines are partly intermediate between the English and Nebraska lines, while in one egg production was more rapid than in the English line, in others slower than in the Nebraska line.

In F_3 , many lines laid eggs more slowly than the Nebraska line, none as rapidly as the English line. The great variation in the results does not permit of any significant classification of the various lines. The fact that the mean number of eggs per

TABLE 11

Showing the distribution of the eggs of all lines of *Hydatina senta* described in this paper, with respect to the time of development of their eggs

GENERATION	LINE	DATE	TIME OF EGG DEVELOPMENT, IN HOURS															
			12	13	14	15	16	17	18	19	20	21	22	23	24			
P ₁	Eng.	July.....		21	23	15	10	6	4	2	3	1						
		Nov.....			3	6	9	16	8	5	0	4						
		Dec.....			1	3	7	6	8	4	2	3	1					
	Neb.	Mar.-Apr.....			2	6	4	10	11	9	4	1	1					
		July.....	18	75	21	19	1											
		Nov.....		2	10	28	14											
		Dec.....		1	16	38	19	9										
		Mar.-Apr.....			1	20	18	15	8									
F ₁	80	Dec.....					4	14	7	7	1	0	1					
	81	Mar.-Apr.....					6	14	14	7	9	6	3	1				
	44	July.....	1	19	27	24	19	1	3	2	0	4						
	47	July.....		16	20	35	14	4	0	2	1	1						
F ₂	50	July.....		14	14	18	17	8	2	0	1							
	51	July.....		7	7	23	11	3	6	2	0	1						
	54	July.....		11	52	42	20	10	3	1								
	55	July.....			12	10	1	1	2	4								
	56	July.....		7	9	7	1	5	2	0	2	1						
	57	July.....		3	8	9	13	3	1	1								
F ₃	58	Nov.....				1	9	14	2	2	0	2						
	59	Nov.....			1	2	5	11	7	0	1							
	60	Nov.....				8	10	8	3	5	4	0	1					
	61	Nov.....			1	1	12	16	4	4	0	2						
	62	Nov.....			2	1	10	8	3	6	4	1						
	63	Nov.....			1	3	16	6	4	6	0	1	2					
	66	Nov.....			2	1	10	8	7	3	3	0	1					
	67	Nov.....			1	0	9	6	6	3	0	1						
	68	Nov.....				3	12	6	6	7	1	0	2					
	69	Nov.....			1	2	12	11	1	0	1	1						
	70	Nov.....					18	20	12	1	1	0	3					
	71	Nov.....			1	4	16	10	0	3	1	3						
	72	Nov.....				6	10	6	2	0	2	1	1					
	64	Nov.....				1	14	4	4	3	0	1						
	65	Nov.....				1	0	1	2	1								
F ₄	73	Nov.....			1	0	6	10	6	1	0	2						
	74	Dec.....				2	9	13	7	2	4	0						
	75	Dec.....					6	14	8	1	3	1	2	0	1			
	76	Dec.....				2	2	8	5	4	2	1	2	1	3			
	77	Dec.....				1	4	32	12	7	4	1	0	1				
	78	Dec.....						5	2	0	1	1						
	79	Dec.....					3	11	9	7	0	3	0	1				

TABLE 11—Continued

GENERA- TION	LINE	DATE	TIME OF EGG DEVELOPMENT, IN HOURS															
			12	13	14	15	16	17	18	19	20	21	22	23	24			
F ₃ back- cross	82	Mar.-Apr.....				1	2	7	8	3	4	2	1	1				
	83	Mar.-Apr.....					3	5	9	8	6	6	1	0	1			
	84	Mar.-Apr.....					6	8	10	6	5	2	2	2				
	85	Mar.-Apr.....						3	0	3	2	0	1	1				
	86	Mar.-Apr.....				1	1	10	2	6	3	4	3	2				
F ₄ from back- cross	87	Mar.-Apr.....				1	2	7	7	5	7	3	1	2				
	88	Mar.-Apr.....					4	3	9	13	9	5	1					
	89	Mar.-Apr.....					4	14	14	6	12	6	3	2				
	108	Mar.-Apr.....				1	2	4	4	3	2	2	3	1				
	90	Mar.-Apr.....					8	11	10	10	10	5	2					
	91	Mar.-Apr.....					3	9	14	3	5	4	3	1				
	92	Mar.-Apr.....					2	8	16	14	11	2	2					
	93	Mar.-Apr.....					2	6	9	13	14	11	5	4				
	94	Mar.-Apr.....		1	10	13	14	9	6	4	3							
	95	Mar.-Apr.....					3	6	12	12	7	1	2	1				
	96	Mar.-Apr.....				1	3	4	8	7	8	4	1	1				
	97	Mar.-Apr.....						2	4	3	2	0	1					
	98	Mar.-Apr.....				3	2	4	9	3	5	4	2					
	99	Mar.-Apr.....				2	1	7	8	5	7	1						
	100	Mar.-Apr.....					2	5	6	11	5	0	1	0	1			
	102	Mar.-Apr.....				1	2	5	20	14	10	5	1					
	103	Mar.-Apr.....							16	28	43	19	10					
	104	Mar.-Apr.....					1	1	2	2	0	2	1					
	105	Mar.-Apr.....				1	3	6	10	13	5	6	1	1				
	106	Mar.-Apr.....					2	9	7	9	6	6	1	1				
	107	Mar.-Apr.....				1	3	2	5	5	4	0	3					
	101	Mar.-Apr.....					1	3	19	12	8	0	2	1				

day for all the F₃ lines is a trifle less than the number per day for the Nebraska line in November, may be due to a loss of vigor resulting from inbreeding. I have shown elsewhere (Shull '12) that inbreeding results in a decrease of vigor in these rotifers. Lines 58 and 65, in particular, showed other evidences of weakness and were lost before any of the other F₃ lines. As shown in table 13, they were among the slowest egg producers.

No safe conclusions regarding inheritance of the rate of egg production can be drawn from the data obtained.

TABLE 12

Showing mean time of development, and the variability of time of development, of the lines of Hydatina senta described in this paper, as computed from data in table 11

GENERATION	LINE	DATE	NO. OF EGGS	MEAN TIME OF DEVELOPMENT, IN HOURS	STANDARD DEVIATION OF TIME OF DEVELOPMENT	EXCESS OF MEAN OVER THAT OF ENGLISH LINE AT SAME DATE	EXCESS OF STANDARD DEVIATION OVER THAT OF ENGLISH LINE AT SAME DATE
P ₁	Eng.	July.....	85	14.98±0.21	1.94±0.15		
		Nov.....	51	17.08±0.24	1.72±0.17		
		Dec.....	35	17.66±0.33	1.94±0.23		
		Mar.-Apr..	44	18.55±0.28	1.87±0.20		
	Neb.	July.....	134	13.33±0.08	0.90±0.06	-1.65±0.22	-1.04±0.16
		Nov.....	54	15.00±0.10	0.77±0.07	-2.08±0.26	-0.95±0.18
		Dec.....	83	15.23±0.10	0.92±0.07	-2.43±0.34	-1.02±0.24
		Mar.-Apr..	62	16.15±1.14	1.06±0.10	-2.40±0.31	-0.81±0.22
F ₁	80	Dec.....	34	17.74±0.22	1.27±0.15	+0.08±0.40	-0.67±0.27
	81	Mar.-Apr..	60	18.57±0.27	2.09±0.19	+0.02±0.39	+0.22±0.28
	44	July.....	100	14.72±0.18	1.82±0.13	-0.26±0.28	-0.12±0.20
	47	July.....	93	14.88±0.15	1.49±0.11	-0.10±0.26	-0.45±0.19
F ₂	50	July.....	75	15.03±0.17	1.47±0.12	+0.05±0.27	-0.47±0.19
	51	July.....	60	15.47±0.22	1.67±0.15	+0.49±0.30	-0.27±0.21
	54	July.....	139	14.85±0.10	1.20±0.07	-0.13±0.23	-0.74±0.17
	55	July.....	30	15.43±0.32	1.76±0.23	+0.45±0.38	-0.18±0.27
	56	July.....	34	15.29±0.37	2.16±0.26	+0.31±0.43	+0.22±0.30
	57	July.....	38	15.32±0.21	1.32±0.15	+0.34±0.30	-0.62±0.21
F ₃	58	Nov.....	30	17.10±0.25	1.35±0.17	+0.02±0.35	-0.37±0.24
	59	Nov.....	27	16.93±0.23	1.18±0.16	-0.15±0.33	-0.54±0.25
	60	Nov.....	39	17.10±0.29	1.79±0.20	+0.02±0.38	+0.07±0.26
	61	Nov.....	40	17.08±0.22	1.39±0.16	0.00±0.33	-0.33±0.25
	62	Nov.....	35	17.37±0.24	1.43±0.17	+0.29±0.34	-0.29±0.24
	63	Nov.....	39	17.13±0.29	1.83±0.20	+0.05±0.38	+0.11±0.27
	66	Nov.....	35	17.26±0.29	1.71±0.20	+0.18±0.38	-0.01±0.26
	67	Nov.....	26	17.15±0.28	1.41±0.20	+0.07±0.37	-0.31±0.26
	68	Nov.....	37	17.41±0.28	1.73±0.20	+0.33±0.37	+0.01±0.26
	69	Nov.....	29	16.97±0.26	1.38±0.18	-0.11±0.35	-0.34±0.25
	70	Nov.....	55	17.25±0.19	1.42±0.14	+0.17±0.31	-0.30±0.22
	71	Nov.....	38	16.84±0.27	1.69±0.19	-0.24±0.36	-0.03±0.25
	72	Nov.....	32	16.84±0.03	1.71±0.21	-0.24±0.38	-0.01±0.27
	64	Nov.....	27	16.93±0.26	1.36±0.19	-0.15±0.35	-0.36±0.25
	65	Nov.....	5	17.40±0.61	1.36±0.43	+0.32±0.65	-0.36±0.46
F ₄	73	Nov.....	26	17.27±0.29	1.46±0.20	+0.19±0.38	-0.26±0.26
	74	Dec.....	42	17.98±0.35	2.30±0.25	+0.32±0.48	+0.36±0.34
	75	Dec.....	36	17.94±0.31	1.88±0.22	+0.28±0.45	-0.06±0.32
	76	Dec.....	30	18.80±0.47	2.59±0.33	+1.14±0.57	+0.65±0.40
	77	Dec.....	62	17.68±0.17	1.32±0.12	+0.02±0.37	-0.62±0.26
	78	Dec.....	9	18.00±0.47	1.41±0.33	+0.34±0.57	-0.53±0.40
	79	Dec.....	34	18.12±0.27	1.55±0.19	+0.46±0.43	-0.39±0.30

TABLE 12—Continued

GENERATION	LINE	DATE	NO. OF EGGS	MEAN TIME OF DEVELOPMENT, IN HOURS	STANDARD DEVIATION OF TIME OF DEVELOPMENT	EXCESS OF MEAN OVER THAT OF ENGLISH LINE AT SAME DATE	EXCESS OF STANDARD DEVIATION OVER THAT OF ENGLISH LINE AT SAME DATE
F ₃ back cross	82	Mar.-Apr..	29	18.41±0.34	1.83±0.24	-0.14±0.44	-0.04±0.31
	83	Mar.-Apr..	39	18.95±0.28	1.75±0.20	+0.40±0.40	-0.12±0.28
	84	Mar.-Apr..	41	18.49±0.30	1.90±0.21	-0.06±0.41	+0.03±0.29
	85	Mar.-Apr..	10	19.30±0.62	1.95±0.50	+0.75±0.68	+0.08±0.54
	86	Mar.-Apr..	32	18.97±0.38	2.13±0.27	+0.42±0.47	+0.26±0.34
F ₄ from back cross	87	Mar.-Apr..	35	18.80±0.34	1.91±0.24	+0.25±0.44	+0.04±0.31
	88	Mar.-Apr..	44	18.89±0.22	1.47±0.16	+0.34±0.36	-0.40±0.26
	89	Mar.-Apr..	61	18.79±0.23	1.80±0.17	+0.24±0.36	-0.07±0.26
	108	Mar.-Apr..	22	18.86±0.50	2.20±0.35	+0.31±0.57	+0.33±0.40
	90	Mar.-Apr..	56	18.46±0.22	1.68±0.16	-0.09±0.36	-0.19±0.26
	91	Mar.-Apr..	42	18.64±0.28	1.80±0.20	+0.09±0.40	-0.07±0.28
	92	Mar.-Apr..	55	18.69±0.18	1.33±0.13	+0.14±0.33	-0.54±0.24
	93	Mar.-Apr..	64	18.63±0.22	1.74±0.16	+0.08±0.36	-0.13±0.26
	94	Mar.-Apr..	60	17.15±0.22	1.72±0.16	-1.40±0.36	-0.15±0.26
	95	Mar.-Apr..	44	18.68±0.23	1.53±0.17	+0.13±0.36	-0.34±0.26
	96	Mar.-Apr..	37	18.87±0.29	1.77±0.21	+0.32±0.40	-0.10±0.29
	97	Mar.-Apr..	12	18.75±0.39	1.36±0.28	+0.20±0.48	-0.51±0.34
	98	Mar.-Apr..	32	18.50±0.34	1.95±0.24	-0.05±0.44	+0.08±0.31
	99	Mar.-Apr..	31	18.23±0.27	1.50±0.19	-0.32±0.39	-0.37±0.28
	100	Mar.-Apr..	31	18.71±0.29	1.61±0.21	+0.16±0.40	-0.26±0.29
	102	Mar.-Apr..	58	18.71±0.18	1.37±0.13	+0.16±0.33	-0.50±0.24
	103	Mar.-Apr..	116	18.82±0.10	1.13±0.07	+0.27±0.30	-0.74±0.21
	104	Mar.-Apr..	9	19.00±0.63	1.89±0.45	+0.45±0.69	+0.02±0.49
	105	Mar.-Apr..	46	18.76±0.25	1.67±0.18	+0.21±0.38	-0.20±0.27
	106	Mar.-Apr..	41	18.85±0.26	1.68±0.18	+0.30±0.38	-0.19±0.27
	107	Mar.-Apr..	23	18.61±0.40	1.91±0.28	+0.06±0.49	+0.04±0.34
	101	Mar.-Apr..	46	18.78±0.19	1.32±0.13	+0.23±0.34	-0.55±0.24

Inheritance of place of egg laying

The hereditary relations of the various lines, with regard to the place of egg laying, appear to be rather definite. Table 14 gives the results obtained from all lines except 48, 49, 95 to 99, and 101, which were lost before the location of the eggs could be determined. Notice should be taken of the different results in the original English and Nebraska lines in different months, when the results in the filial lines are judged.

From July to December, all filial lines except 65, 71, 73, 75, 76, and 79, laid from 50 to 60 per cent of their eggs at the surface film; and the six lines just named varied so little above or below these limits that the difference is fairly chargeable to fluctuation. All the lines of the first four filial generations, therefore, are closely similar to the English line. In the period March to June, the

TABLE 13

Showing the number of eggs laid per day by the females of the lines of Hydatina senta described in this paper, through the F₃ generation and part of F₄

GENERATION	LINE	DATE	NUMBER OF FEMALES LAYING	NUMBER OF INDIVIDUAL DAYS OF LAYING	NUMBER OF EGGS LAID	NUMBER OF EGGS PER DAY PER FEMALE
P ₁	Eng.	July-Aug.....	14	27.86	418	15.0
		Nov.....	12	13.80	141	10.2
	Neb.	July-Aug.....	22	34.54	420	12.2
		Nov.....	23	23.40	160	6.8
F ₁	44	July.....	4	10.10	158	15.6
	47	July.....	4	12.60	167	13.2
F ₂	50	July.....	4	11.10	178	16.0
	51	July.....	4	10.60	123	11.6
	54	July.....	4	12.60	188	14.9
	55	Aug.....	6	13.60	151	11.1
	56	Aug.....	6	7.40	95	12.8
	57	Aug.....	6	15.50	224	14.4
F ₃	58	Nov.....	16	16.00	70	4.4
	59	Nov.....	17	17.20	112	6.5
	60	Nov.....	16	14.80	125	8.4
	61	Nov.....	20	21.40	143	6.6
	62	Nov.....	18	18.80	139	7.4
	63	Nov.....	16	17.40	129	7.4
	66	Nov.....	10	10.80	97	9.0
	67	Nov.....	15	14.80	136	9.2
	68	Nov.....	9	8.50	32	3.8
	69	Nov.....	15	14.60	66	4.5
	70	Nov.....	17	17.70	147	8.3
	71	Nov.....	13	13.60	126	9.3
	72	Nov.....	17	17.50	127	7.3
	64	Nov.....	14	14.90	70	4.7
	65	Nov.....	10	9.80	32	3.3
F ₄	73	Nov.....	13	13.4	91	6.8

numbers of eggs at the surface were higher in all lines, including the parental (English and Nebraska) lines. While none of the back cross lines, nor those of the F_4 generation derived from the back cross, laid quite as many eggs at the surface as did the English line during the same period, the difference is not certainly significant except in line 103. In line 103, only 35.5 per cent of the eggs were at the surface film. For reasons given (pp. 154-156), I know of no way to determine how small a difference, in the present case, is to be regarded as significant. But in view of the large number of eggs counted in line 103, a difference of more than 50 per cent (88.9 to 35.5) can hardly be considered meaningless. At the same time, the number of eggs at the surface is not as low as in the Nebraska line.

To summarize: line 103, of the F_4 generation from the back cross, is the only line showing any strong indication of inheritance from the Nebraska line, with regard to the place of egg laying.

Inheritance of contractility of foot muscles

Contractility was in all cases determined by killing the females within 24 hours after egg laying began, in Bouin's fluid. The method was in all particulars the same as given on page 157 for determining the difference between the English and Nebraska lines. Lines 48, 49, 91 and 95 to 99, inclusive, were lost before the contractility of the foot muscles could be tested.

The results, as shown in table 15, are briefly stated as follows: Of all the filial lines, only five showed greater contractility of the foot muscles than the English line. These five are the ones to which the *plus* sign is prefixed in the last column of table 15. In four of the five, the excess of the degree of contractility (mean class number) over that of the English line is less than its mean error. The remaining one, line 93, shows a degree of foot retraction that must be regarded as significant. Line 93, therefore, is the only filial line that gives certain indication of inheritance from the Nebraska line, with regard to the contraction of the foot muscles.

The fact that nearly all mean class numbers in the filial lines are slightly less than the mean of the English line may be due

TABLE 14

Showing number of eggs laid at bottom of dish and at surface film of water, by the lines of Hydatina senta described in this paper

GENERATION	LINE	DATE	NUMBER OF EGGS AT SURFACE	NUMBER OF EGGS AT BOTTOM	PERCENTAGE OF EGGS AT SURFACE
P ₁	Eng.	July-Dec.....	831	706	54.1
		June.....	370	46	88.9
	Neb.	July-Dec.....	118	1067	9.9
		Mar.-June.....	120	439	21.4
F ₁	80	July-Dec.....	436	340	56.1
	81	July-Dec.....	380	341	52.7
	44	July-Dec.....	913	703	56.1
	47	July-Dec.....	781	665	54.0
F ₂	50	July-Dec.....	898	675	57.0
	51	July-Dec.....	942	817	53.4
	54	July-Dec.....	978	682	58.9
	55	July-Dec.....	571	382	59.9
	56	July-Dec.....	926	703	56.8
	57	July-Dec.....	872	697	55.5
F ₃	58	July-Dec.....	201	194	50.8
	59	July-Dec.....	248	217	53.3
	60	July-Dec.....	204	166	55.1
	61	July-Dec.....	214	190	52.9
	62	July-Dec.....	238	189	55.7
	63	July-Dec.....	182	122	59.8
	66	July-Dec.....	176	144	55.0
	67	July-Dec.....	178	160	52.6
	68	July-Dec.....	219	194	53.0
	69	July-Dec.....	153	124	55.2
	70	July-Dec.....	129	103	55.6
	71	July-Dec.....	276	177	60.9
	72	July-Dec.....	231	196	54.0
	64	July-Dec.....	191	179	51.6
	65	July-Dec.....	78	85	47.8
F ₄	73	July-Dec.....	123	54	69.4
	74	July-Dec.....	490	425	53.5
	75	July-Dec.....	317	347	47.7
	76	July-Dec.....	341	349	49.9
	77	July-Dec.....	378	340	52.6
	78	July-Dec.....	143	107	57.2
	79	July-Dec.....	260	284	47.7

TABLE 14—Continued

GENERATION	LINE	DATE	NUMBER OF EGGS AT SURFACE	NUMBER OF EGGS AT BOTTOM	PERCENTAGE OF EGGS AT SURFACE
F ₃ back cross	82	Mar.-Apr.	355	106	77.0
	83	Mar.-Apr.	363	100	78.4
	84	Mar.-Apr.	450	154	74.5
	85	Mar.-Apr.	234	41	85.0
	86	Mar.-Apr.	490	87	84.9
F ₄ from back cross	87	Mar.-Apr.	267	54	83.2
	88	Mar.-Apr.	422	110	79.3
	89	Mar.-Apr.	216	50	81.2
	108	June.	215	76	73.9
	90	Mar.-Apr.	323	71	81.9
	91	Mar.-Apr.	275	69	79.9
	92	Mar.-Apr.	310	82	79.1
	93	Mar.-Apr.	225	79	74.0
	94	Mar.-Apr.	371	115	76.3
	100	June.	204	64	76.1
	102	June.	357	125	74.1
	103	June.	468	852	35.5
	104	June.	236	90	72.4
	105	June.	301	111	73.1
	106	June.	251	98	68.7
	107	June.	326	148	68.7

to a change in judgment regarding the limits of the four arbitrarily selected degrees of contraction, as the investigation proceeded. The differences are so small, compared with their mean errors, that I believe them to be insignificant.

DISCUSSION

Review of results

The results of the experiments above described present certain unexpected features that need to be explained. In table 16 the results are summarized. Variability of the time of egg development, and the rate of egg production, are omitted from this table because of the irregularity of their inheritance. The following remarks pertain only to the four remaining characteristics, which are included in table 16.

TABLE 15

Showing distribution of the females of the lines of Hydatina senta described in this paper, with respect to the degree of contraction of the foot muscles (see text for further description of class numbers)

GENERATION	LINE	NUMBER OF FEMALES SHOWING FOLLOWING DEGREES OF CONTRACTION				TOTAL NO. OF ♀♀	MEAN CLASS NUMBER	STANDARD DEVIATION	EXCESS OF MEAN OVER THAT OF ENGLISH LINE
		1 Fully extended	2 Slightly contracted	3 Moderately contracted	4 Greatly contracted				
P ₁	Eng.	34	13	3	1	51	1.39±0.101	0.72±0.071	
	Neb.	1	5	25	81	112	3.66±0.057	0.61±0.040	+2.27±0.166
F ₁	80	100	17	14	2	133	1.38±0.063	0.73±0.045	-0.01±0.119
	81	95	18	6	2	121	1.30±0.058	0.64±0.041	-0.09±0.116
	44	36	16	5	1	58	1.50±0.096	0.73±0.068	+0.11±0.139
	47	70	26	0	5	101	1.41±0.072	0.73±0.051	+0.02±0.124
F ₂	50	43	9	0	2	54	1.28±0.088	0.65±0.062	-0.11±0.134
	51	38	10	2	2	52	1.38±0.102	0.74±0.072	-0.01±0.144
	54	45	7	1	3	55	1.33±0.102	0.76±0.072	-0.06±0.144
	55	43	12	1	0	56	1.25±0.063	0.47±0.045	-0.14±0.119
	56	33	7	1	0	41	1.22±0.073	0.47±0.052	-0.17±0.125
	57	37	9	1	1	48	1.29±0.088	0.61±0.062	-0.10±0.134
F ₃	58	35	11	3	1	50	1.40±0.097	0.69±0.069	+0.01±0.140
	59	45	9	3	2	59	1.36±0.095	0.73±0.067	-0.03±0.139
	60	38	7	1	2	48	1.31±0.102	0.71±0.072	-0.06±0.144
	61	107	21	3	1	132	1.23±0.045	0.52±0.032	-0.16±0.112
	62	70	11	3	2	86	1.27±0.069	0.64±0.049	-0.12±0.122
	63	71	11	1	3	86	1.26±0.070	0.65±0.049	-0.13±0.123
	66	83	16	10	4	113	1.42±0.075	0.80±0.053	+0.03±0.126
	67	85	17	5	0	107	1.25±0.051	0.53±0.036	-0.14±0.113
	68	114	15	7	1	137	1.23±0.048	0.57±0.034	-0.16±0.112
	69	67	15	3	1	86	1.28±0.063	0.58±0.045	-0.11±0.119
	70	49	11	1	0	61	1.21±0.058	0.45±0.041	-0.18±0.117
	71	88	14	1	2	105	1.21±0.054	0.55±0.038	-0.18±0.115
	72	34	16	0	0	50	1.32±0.066	0.47±0.047	-0.07±0.121
	64	71	7	4	2	84	1.25±0.070	0.64±0.049	-0.14±0.123
	65	48	3	2	2	55	1.24±0.093	0.69±0.066	-0.15±0.137

TABLE 15—Continued

GENERA- RATION	LINE	NUMBER OF FEMALES SHOWING FOLLOWING DE- GREES OF CONTRACTION				TOTAL NO. OF ♀ ♀	MEAN CLASS NUMBER	STANDARD DEVIATION	EXCESS OF MEAN OVER THAT OF ENGLISH LINE
		1 Fully extended	2 Slightly contracted	3 Moderately contracted	4 Greatly contracted				
F ₄	73	65	4	1	2	72	1.17±0.069	0.58±0.049	-0.22±0.122
	74	71	16	5	1	93	1.31±0.064	0.62±0.045	-0.08±0.120
	75	65	8	4	3	80	1.31±0.082	0.74±0.058	-0.08±0.130
	76	83	15	0	1	99	1.18±0.046	0.46±0.033	-0.21±0.111
	77	72	10	0	0	82	1.12±0.036	0.33±0.025	-0.27±0.107
	78	87	20	3	1	110	1.26±0.052	0.55±0.037	-0.13±0.113
	79	65	11	4	0	80	1.24±0.059	0.53±0.042	-0.15±0.117
F ₃ back cross	82	83	11	5	1	100	1.24±0.059	0.59±0.042	-0.15±0.117
	83	90	5	3	2	100	1.22±0.055	0.55±0.039	-0.17±0.115
	84	79	16	5	0	100	1.26±0.054	0.54±0.038	-0.13±0.115
	85	53	8	3	0	64	1.22±0.064	0.51±0.045	-0.17±0.119
	86	50	8	4	2	64	1.34±0.093	0.74±0.066	-0.05±0.137
F ₄ from back cross	87	85	10	4	1	100	1.21±0.055	0.55±0.039	-0.18±0.115
	88	65	10	3	3	81	1.31±0.079	0.71±0.056	-0.08±0.128
	89	79	19	2	0	100	1.23±0.047	0.47±0.033	-0.16±0.111
	108	79	18	2	1	100	1.25±0.054	0.54±0.038	-0.14±0.115
	90	84	10	4	2	100	1.24±0.062	0.62±0.044	-0.15±0.119
	92	81	12	4	3	100	1.29±0.068	0.68±0.048	-0.10±0.122
	93	76	52	40	28	196	2.10±0.076	1.07±0.054	+0.71±0.126
	94	78	28	2	0	108	1.30±0.047	0.49±0.033	-0.09±0.111
	100	86	10	3	1	100	1.19±0.052	0.52±0.037	-0.20±0.114
	102	79	13	6	2	100	1.31±0.067	0.67±0.047	-0.08±0.121
	103	81	12	5	2	100	1.28±0.068	0.68±0.048	-0.11±0.122
	104	83	15	2	0	100	1.21±0.043	0.43±0.030	-0.18±0.110
	105	76	20	4	0	100	1.28±0.053	0.53±0.037	-0.11±0.114
	106	80	14	4	2	100	1.28±0.063	0.63±0.045	-0.11±0.119
	107	75	24	1	0	100	1.26±0.046	0.46±0.033	-0.13±0.111
	101	80	14	5	1	100	1.27±0.060	0.60±0.042	-0.12±0.117

TABLE 16

Summary of the results of the experiments, showing in particular the similarity of the filial lines to the English parent line

CHARACTER	GENERATION					
	F ₁	F ₂	F ₃	F ₄	F ₃ back-cross	F ₄ from back-cross
Size of egg	All 3 like Eng.	All 6 like Eng.	14 lines like Eng., 1 (61) intermediate	5 like Eng., 1 (75) intermediate 1 (79) perhaps a little larger than Eng.	All 5 like Eng.	8 lines like Eng. 2 (103, 107) like Neb. 4 (90, 93, 94, 106) perhaps larger than Neb. 2 (108, 100) doubtful
Time of egg development	Both like Eng.	All 6 like Eng.	All 15 like Eng.	All 7 like Eng.	All 5 like Eng.	21 lines like Eng.
Place of egg laying	Both like Eng.	All 6 like Eng.	All 15 like Eng.	All 7 like Eng.	All 5 like Eng.	1 (94) intermediate 15 lines like Eng.
Contractility of foot muscles	Both like Eng.	All 6 like Eng.	All 15 like Eng.	All 7 like Eng.	All 5 like Eng.	1 (103) intermediate 15 lines like Eng. 1 (93) intermediate

In the several crosses obtained in F₁, each line possessed the four characteristics of the English line. This statement applies to both reciprocal crosses. It is rather curious, though not impossible, that all the dominant characteristics should have belonged to one of the original lines.

In F₂, where segregation and the reappearance of the characteristics of the Nebraska line might be expected, all of the lines again showed only the traits of the English rotifers.

In F₃, with a single exception, all the lines again repeated the four characters of the English line. The one exception is line 61, which, in egg size, was intermediate between the English and Nebraska lines, but like the English rotifers in other respects. It is important to note, as shown in table 9, that line 61 is descended from the same source (line 56) as twelve other lines in F₃. The fact that none of the other lines in F₃ showed any evidence of segregation can not, therefore, be attributed to the possibility that all the other lines descended from a parent line that was homozygous for each dominant character. If line 56 could transmit greater size of egg to line 61, there were twelve other F₃ lines in which larger eggs would have been possible.

In F_4 , the seven lines were again all like the English line, with the exception of one line (75), and perhaps a second (79). These two lines differed from the English line in size of egg, though perhaps not significantly in line 79. These two exceptional lines are descended, as table 9 shows, from line 61, which was itself the only exceptional line in F_3 .

In the back crosses there was no line that differed significantly from the English line in any of the four characters; and this was true, notwithstanding that line 56, ancestor of lines 61, 75, and 79, with their larger eggs, was used in making the back cross.

In the F_4 generation obtained by inbreeding the lines of the back cross, there was more evidence of segregation, though less than would be expected if the characters studied behaved in regular Mendelian fashion (see table 16).

Viewing the results as a whole, there was much less indication of segregation than might be expected. Furthermore, the four characters appear on the whole to have been transmitted as a group. Where segregation failed in one character, it usually failed in the rest. The departures from this rule are few in number, except in the last generation obtained. The importance of these exceptions is pointed out in what follows.

Explanations of results

Did crossing actually occur? The results in the early filial generations, in which all the lines were like the English parent line in all the characters studied, were what would have been expected if the eggs from which they originated had been parthenogenetic eggs or inbred fertilized eggs, of the English line, instead of cross fertilized eggs. It is not impossible that eggs more or less similar to the fertilized eggs may nevertheless be unfertilized. The fertilized eggs are, on the average, larger than the parthenogenetic eggs, and have a thicker shell set with pilose projections. I have shown elsewhere (Shull '10, p. 343) that parthenogenetic eggs may have thicker shells than usual, following the impregnation of the female that lays them. In the case described in my earlier paper, just mentioned, a sexual female had been

impregnated, and laid two large fertilized eggs, one of which hatched as a female. The same impregnated sexual female then laid numerous small eggs, all of which had somewhat thicker shells than male eggs, and required a somewhat longer time to develop, but which yielded males, showing them to have been parthenogenetic. If the thickening of the shell were carried further, the eggs might readily be classed as fertilized, and yet be unfertilized.

In view of the possibility that the eggs in these experiments were not truly fertilized, it seems best to state some facts which appear to me to exclude both parthenogenesis and inadvertent inbreeding.

In making the crosses, males of the one line were placed in a dish with young females and unhatched eggs of the other line. Before the females were half grown, and hence *before they could have produced any males of their own line*, each female was isolated in a dish and reared to maturity. This method, if carefully followed, insures that whatever fertilized eggs these females lay are cross fertilized eggs.

Lest, however, some error might have crept in, we may examine the internal evidence of crossing.

Line 44, derived from a cross between a Nebraska female and an English male, should, in case it was produced *either* parthenogenetically or by an inbred Nebraska female, have been like the Nebraska line. Instead, it was like the English line. And lines 51 and 54, descendants from line 44 by inbreeding, were likewise similar to the English line in all four characters.

Line 47 (an F_1) yielded many more male-producers than either of the original lines. Had it been produced parthenogenetically, the proportion of male-producers should have been the same as in the line that produced it.

The rate of egg laying and general vigor of the females varied in the several F_1 lines. Had they been produced parthenogenetically they should all have been alike.

In view of these internal evidences of crossing, and the method of making the crosses, there can be no doubt, it seems to me, that the lines described in this paper were produced by true crosses

between the English and Nebraska lines. This conclusion is strongly supported by the results in the F_4 generation, and F_4 from the back cross, results which can be best explained as due to inheritance from the Nebraska line.

Typical Mendelian behavior a possibility. The curious results summarized at the beginning of the discussion may, with the aid of an assumption regarding physiological facts, have a truly Mendelian explanation. Suppose each of the four characters in question were a simple Mendelian character. The original English and Nebraska lines were probably homozygous with respect to all four characters; the few results obtained by inbreeding them (see lines 48, 80, 81) are what would be expected if they were homozygous, and all other lines collected with them, in England and Nebraska, respectively, were like them at least in egg size. Moreover, there is no internal evidence in the experiments to show that they were other than homozygous.

If the original lines were homozygous, the three hybrid lines in F_1 were heterozygous in all four characters. Females of such lines should produce sixteen kinds of gametes, and there should be 256 different combinations in F_2 . One of the 256 should be homozygous for all the characters of the English line, one homozygous for all the characters of the Nebraska line. Of the remainder, 80, or over 31 per cent, should have received each of the characters of the English line from at least one parent. If—and here is the physiological assumption—only those fertilized eggs containing factors for all the characters of the English line could hatch and produce viable lines, the results may be explained. As may be seen from table 9, in no generation was the viability of the fertilized eggs as high as 30 per cent.

The chief objection to this view is found in the fact that a few of the lines did not possess all of the characters of the English line. Lines 90, 106, and 107 were like the English rotifers in only three of the four characteristics; lines 93, 94 and 103 were unlike the English line in two of the four characters; yet these lines were healthy.

The Mendelian explanation becomes easier if we assume that the characters studied were not simple, but dependent upon, to

use the terminology of G. H. Shull ('14), plural determiners. The proportion of combinations in F_2 , for example, which would include all the characters of the English line, would be increased by the presence of plural determiners for each character, the amount of increase depending on the number of plural factors. The fertilized eggs that hatched might well have given rise, under these circumstances, to lines like the English line in all four characters, purely as a matter of chance, without the assumption that mortality was selective.

The probability that the eggs that hatched would produce lines wholly like the English line would be still greater if the plural determiners for each character were also, again employing the terminology of G. H. Shull, duplicate determiners (that is, factors, each of which alone produces a character indistinguishable from that produced by any of the remaining duplicate determiners) and fully dominant. I do not think this condition is probable in the case of the four characters here studied, partly because, as Shull (loc. cit) has pointed out, very few cases of duplicate genes have yet been demonstrated, and partly because the unequal intermediate characters appearing in several lines (see those of F_3 , F_4 , and F_4 from the back cross) are better explained as due to (perhaps) unequal and cumulative determiners.

The four characters perhaps identical. At the end of the F_2 generation, and before the data in F_3 were all collected, the fact that the four characters seemed to go together in the same lines suggested the possibility that the four characters were but different manifestations of a single character. At first I tried to relate all of them to size of body. As stated on page 157, the bodies of the Nebraska females were somewhat larger than those of the English females. Naturally, I supposed, their eggs should be larger, and perhaps the size influenced the time of development. Larger bodies might have induced the females to remain mostly at the bottom, and so lay their eggs there. And lastly, it seemed possible, as explained on page 157, that the contraction of the foot muscles might be due to mechanical stimulus dependent on large bodies.

This hypothesis was soon abandoned, because, as recounted on page 157, the contraction of the foot muscles was proven not to be due to size of body. Dr. O. C. Glaser suggested that the single difference at the basis of the four described differences between the two lines, might be a difference in permeability; that, in some way, larger eggs, quicker development of eggs, laying eggs at the bottom of the water, and greater contractility of the foot muscles, might be due to a greater or less degree of permeability. Some brief experiments to test this possibility have been described on page 157. They resulted negatively, that is, both lines appeared to be equal in permeability; but I hope further tests may be made.

Serious obstacles to any explanation by which the four apparently distinct characters are combined into one, are found in those lines in F_3 , in F_4 and especially in F_4 from the back cross, in which the four characters *do not go together*. Egg size is repeatedly shown to be more or less independent of the other three characters (lines 61, 75, 90, 106, 107). Short time of development of the eggs may separate, to some extent, from egg laying at the bottom and high contractility of the foot muscles (line 94). Egg laying at the bottom may, to a degree, part company with great contractility of muscles and short time of egg development (line 103). Great contractility of muscles need not occur in the same line with egg laying at the bottom and short time of egg development (line 93).

If the four visible characters are in reality but one, the exceptions just noted will require subsidiary hypotheses.

Association of factors. Any hypothesis that reduces the number of characters which differentiated the original lines from four or more to one, makes simpler an explanation that is essentially Mendelian. In F_2 , for example, where only one fertilized egg in eight yielded a line of rotifers that could be tested (see table 9), those few lines might by chance easily possess the dominant (single) character. The force of the preceding explanation (identity of the four characters) depended on the fact that it substituted one character for four; the weakness of that explanation

was that in certain lines the four characters were evidently *not* one.

Association of factors has all the advantage, it seems to me, of uniting the four characters into one, under most circumstances, and has the further advantage that the union of the determiners is not an ironclad one. We have been made familiar with association of genes through the work of Morgan and his students (Morgan '10, '11 a, '11 b, '14; Bridges '13; Dexter '12; Sturtevant '13 a, '13 b; and numerous other papers) on the fly *Drosophila*. They assume a form of association that may be occasionally broken. The same kind of association may be present in *Hydatina*; the four characters being ordinarily associated, but with the possibility that one or more of them may sometimes part company with the rest.

What the mechanism of association may be, in *Hydatina*, if association exists, is a question I have not attempted to answer. In *Drosophila*, Morgan and his pupils have held it probable that the genes for all associated characters reside in the same chromosome. The chromosomes in *Drosophila* are few in number, and three—or four (Muller '14)—of them have been designated as the seat of different determiners. In *Hydatina*, on the other hand, the chromosomes are numerous (12 to 15 in the haploid groups) and small, as shown by Whitney ('09). I would hesitate to locate in one of these chromosomes all of the heritable characters of the parthenogenetic lines that have been discovered.

The fact that crossing between the English and Nebraska rotifers was more difficult, as stated on page 158, than has been found true of other lines, may be due to some deep-seated disturbance of the normal process of fertilization in these rotifers, a disturbance which carried with it the association of the characters of the English line.

While the hypothesis of association is not to be stated in detail, and while it must not be strongly maintained, it appears to me at the present time quite possible, and not open to most of the objections which tend to disprove the other possible explanations mentioned above.

SUMMARY

Two parthenogenetic lines of the rotifer *Hydatina senta*, obtained from England and Nebraska, respectively, were found to differ in the following respects:

1. The eggs of the Nebraska line were larger than those of the English line. Measurements to demonstrate this difference were made only upon eggs laid in the first 24 hours of egg laying of any female, because it was shown that the eggs of one female gradually increased in size with increasing age of the mother.

2. The Nebraska eggs developed in about two hours less time, on the average, than did the English eggs. Moreover, the time of development of the Nebraska eggs was much more uniform than that of the English eggs; eggs of the Nebraska females laid at the same time and reared under identical conditions seldom differed from one another, in the time of development, more than an hour, while the extreme times of development of English eggs under like conditions differed by four or five hours.

3. The rate of egg production in the Nebraska line was lower than in the English line, being a little over 12 per day per female in summer in the former line, 15 per day in the latter. Inasmuch as the Nebraska eggs were the larger, the volume of egg substance produced in a given time by a single female was approximately equal in the two lines.

4. More than 50 per cent of the eggs of the English rotifers were laid at the surface film of the water, during the summer; less than 10 per cent of the Nebraska eggs were laid at the surface, the remainder being cemented at the bottom of the dish. The reason for this difference in the location of the eggs is not known; it may be due to a difference in the demand for oxygen. Brief experiments tend to show that the difference in place of egg laying is not due to a difference in permeability. Temperature appears to modify the percentage of eggs laid at the surface.

5. The foot muscles of the Nebraska females responded more vigorously to chemical stimuli than did those of the English

females, so that when the animals were killed in a fixing fluid the foot of the Nebraska females was often retracted completely within the body, that of the English females being much more extended.

Reciprocal crosses were made between the two lines, and the inheritance of the above mentioned characters was traced through six filial generations. The inheritance of the rate of egg production, and of the uniformity of the time of egg development, was too irregular to summarize. No conclusions regarding these two characters are drawn, except that the rate of egg production in the filial lines decreases, probably owing partly to loss of vigor attendant upon inbreeding.

Regarding the four remaining characters (size of egg, time of egg development, place of egg laying, and contractility of the foot muscles) only generalized statements can be made here. Details must be obtained from the discussion and from the description of the experiments.

In F_1 every characteristic of the English line appears to be dominant. In F_2 , there is no evidence of segregation; all the lines show all of the characteristics of the English line. In F_3 , with one exception, all lines are like the English line in each of the four characteristics; the one exception is a line laying eggs of intermediate size, but like the English line in other respects. In F_4 , there is one line (perhaps also a second) that is exceptional in laying eggs intermediate in size, while the other characters of this line, and all four characters of the other lines, were like those of the English line. In a back cross between an F_2 and the Nebraska line, all characters of all lines are again English. In a generation descended by inbreeding from the back cross, there is evidence of segregation of egg size, less evidence of segregation of the factors for the other characters. The results just stated are summarized in tabular form in table 16.

Taking the experiments as a whole, they show much less segregation than was to be expected. The characters of the English line appear to hang together, the transmission of one being the same, with a few exceptions, as that of all the others.

Evidence is given to show that the results of the experiments must be regarded as valid, and several possible explanations of the peculiarities of those results are discussed. The explanation may be typically Mendelian, if selective mortality of the fertilized eggs be assumed, or if each character be represented by plural factors. The view that all four visible differentiating characters are but different manifestations of a single character, is held to be improbable. The hypothesis that the genes for the four characters are associated, but that the associations may be broken, is not strongly advocated; but the objections to it seem less formidable than the objections to the other explanations offered. No mechanism of association is suggested, though it may depend in some way upon an abnormal process of fertilization.

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SIZE DIMORPHISM IN THE SPERMATOOZOA FROM SINGLE TESTES¹

CHARLES ZELENY AND E. C. FAUST

FORTY-THREE FIGURES

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¹ Contribution from the Zoölogical Laboratory of the University of Illinois, No. 36.

INTRODUCTION

Cytological studies have shown that in a large number of species of animals two kinds of spermatozoa are produced, one differing from the other in the nature of its chromatin content. A large mass of indirect evidence makes it highly probable that eggs fertilized by one of these two kinds produce only males and those fertilized by the other only females. Direct experimental test of this hypothesis has not so far been made. As long as the recognition of the differences between the two kinds is confined to the immature stages such a test is not available.

The studies described in the present paper were made with a view to recognition of differences in the mature functional spermatozoa. Where spermatogenesis studies show a difference in amount of chromatin received by the two kinds of spermatids it is natural to expect a corresponding size difference in the heads of the completed spermatozoa. In order to test this hypothesis measurements were made of the sperm-heads in a number of species of animals. A considerable variation in length was found in each case and since expected difference between the two kinds of spermatozoa is small in comparison with the range of variation it is necessary to make a large number of measurements in order to obtain the required size distribution. The plotted curves of variation indicate strongly the presence of size dimorphism in most of the species studied, and confirm the expectation based on the chromosomal histories. Because both expected and observed differences in size are small, and because there are so many factors that may produce apparent dimorphism in the data quite apart from the real dimorphism sought, it was deemed wise to make as many measurements as possible and to test carefully suspected sources of error.^{2,3}

² The first measurements made in October, 1912, disclosed at once a striking dimorphism. The results were discussed by the senior author with some of the zoölogists at the Christmas ('12) meetings at Princeton, and a preliminary paper on *Anasa tristis* was published in the October, 1913, number of the Biological Bulletin (E. C. Faust '13). In the interval there appeared a paper by J. E. Wodsdalek (June '13), describing a similar relation for the spermatozoa of the pig.

³ The authors are indebted to Mr. Charles Hart and Dr. Hugh Glasgow for identification of the insects.

MATERIAL AND METHODS

1. The species chosen for study

Of the available material those species were chosen which seemed to present the fewest difficulties in the way of accurate measurement. In nearly all of these cases the chromosomal history has been worked out and shows the presence of two sets of spermatids, differing from each other in amount of chromatin received. The list of species is given in the table of contents, and details concerning the individual cases are presented under "Data and results" (pp. 201 to 234).

2. Ripeness of spermatozoa

An attempt was made to obtain fully ripe spermatozoa in every case. An examination was made of the living material and it was used only in case spermatozoa with incomplected transformation were absent, and in case rapid movement was general. Motility by itself is not a good criterion of ripeness because movement often appears before the spermatozoa have reached the final shape and size.

3. Preparation of spermatozoa for measurement

The seminal fluid was used either undiluted or diluted with Ringer's solution. It was spread on a slide which had previously been covered with a thin film of egg albumen. Fixation was in osmic fumes, Gilson's mercurio-nitric fluid or Zenker's fluid. Delafield's hematoxylin diluted with two or three volumes of distilled water was used for staining. Several other stains were tried but none proved as satisfactory as the hematoxylin.

The part measured was in every case the chromatin rod of the sperm-head. Attempts were made to include the cytoplasmic portions in the measurements, but it was not possible to get a clear cut outline in the cytoplasm, especially at the anterior end. With Delafield the chromatin stains deeply and under proper conditions with sharp outlines. The cytoplasm stains but slightly in comparison and it is almost impossible to determine its bound-

aries upon the ocular micrometer scale. Since the size differences to be expected are very small it is necessary to have very sharp outlines in the material..

4. Method of measurements

It would be desirable to make measurements of all three dimensions of the sperm-heads, but in most of the spermatozoa studied the width and thickness are so slight that differences of the smallness to be expected could not possibly be distinguished. In one case, however, the bull spermatozoön, both width and length were measured.

The curving of the spermatozoa in many species makes accurate measurement with an ocular micrometer impossible. Only those forms were studied in which all or nearly all of the spermatozoa were straight when fixed. In case curved individuals were present on a slide their lengths were not included.

In taking the measurements it was also necessary to make sure that the head lay horizontally. In no case was a spermatozoön measured unless the whole head was in perfect focus at one time. Measurements were made with an ocular micrometer in a No. 8, 12 or 18 Zeiss compensating ocular, or No. 2 Leitz ocular. A 2 mm. oil immersion objective was used. A mechanical stage is a necessity, to obviate danger of double measurement. The shorter spermatozoa were measured to tenths of an ocular division, the larger ones to tenths, quarters, or halves, though quarters were found to be undesirable and therefore were seldom used. It is well known that there may be a considerable personal error in judging values on a scale even when the observer has no pronounced notion as to expected results. Many tests were therefore made to determine the accuracy of the measurements. These tests involved comparison of the results of measurements of a particular group of spermatozoa by two or three different observers, and numerous remeasurements by single observers.⁴ It was decided from these tests

⁴ Mr. G. F. Sutherland measured several of the sets of spermatozoa as a check upon the determinations made by the authors.

that the accuracy of the method is sufficient for the purpose in view. Because of the necessity of a considerable degree of accuracy the greatest care was taken in many directions. Measurements were not made except when the light was good. Error due to fatigue was avoided by working for short intervals only. In some cases tests were made at the termination of the longer intervals of continued measurement to see if the accuracy suffered because of fatigue. This was done by remeasurement of a number of the spermatozoa last measured. Since the measurement of a single slide of spermatozoa involved many sittings care was taken to make the conditions as regards lighting, fatigue of measurer, etc., as nearly alike as possible at the different times. Furthermore, comparisons made between the measurements at different sittings showed that differences between them are negligible. Tabulations of the data were not made until a whole group was completed. The individual measurements were put down as made, with deliberate effort to avoid comparison. Only after completion of the group was there a tabulation. A considerable experience in making measurements where differences are minute has shown that there is danger either in favoring particular values which are expected to come out ahead in the final tabulation, or else in going to the opposite extreme because of the realization of this danger and the attempt to avoid it. In the latter case, as in the former, one may be distinctly unfair to certain values.

5. Method of interpretation of measurements

If amount of chromatin were the only factor concerned in size there should be two definite and distinct sizes among the completed spermatozoa. In all size determinations it is however true that there is a marked variation in size apart from any known internal factor. Such variations in an otherwise uniform population follow the definite distribution known as the normal variability, or Gaussian distribution (fig. 1). If two populations differing from each other with regard to an internal factor affecting size, but each by itself showing a normal variability distri-

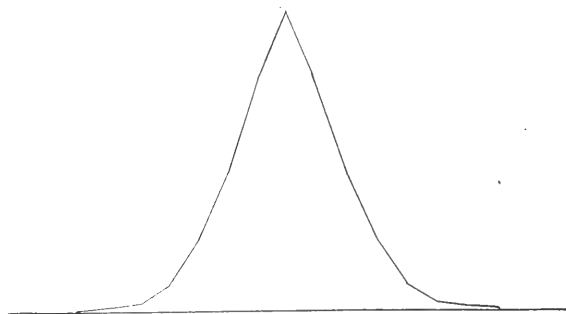


Fig. 1 A population of one thousand individuals exhibiting what is known as a normal variability distribution. The numbers in this figure and in figures 2 to 9 were obtained by the expansion of $(a + b)^{20}$ but a similar distribution is found in many cases of variation:

2 7 22 56 111 181 241 181 111 56 22 7 2

Fig. 2 A population of one thousand individuals consisting of two entirely separate components of five hundred each:

1 3 11 28 55 90 120 90 55 28 11 3 1 — 1 3 11 28 55 90 120
90 55 28 11 3 1

Fig. 3 A population of one thousand individuals consisting of two overlapping components of five hundred each and exhibiting a distribution with two very distinct modes.

Component A	1	3	11	28	55	90	120	90	55	28	11	3	1							
Component B								1	3	11	28	55	90	120	90	55	28	11	3	1
Population	1	3	11	28	55	90	121	93	66	56	66	93	121	90	55	28	11	3	1	

Fig. 4 A population of one thousand individuals consisting of overlapping components of five hundred each and exhibiting a distribution with the two modes not as prominent as in figure 3.

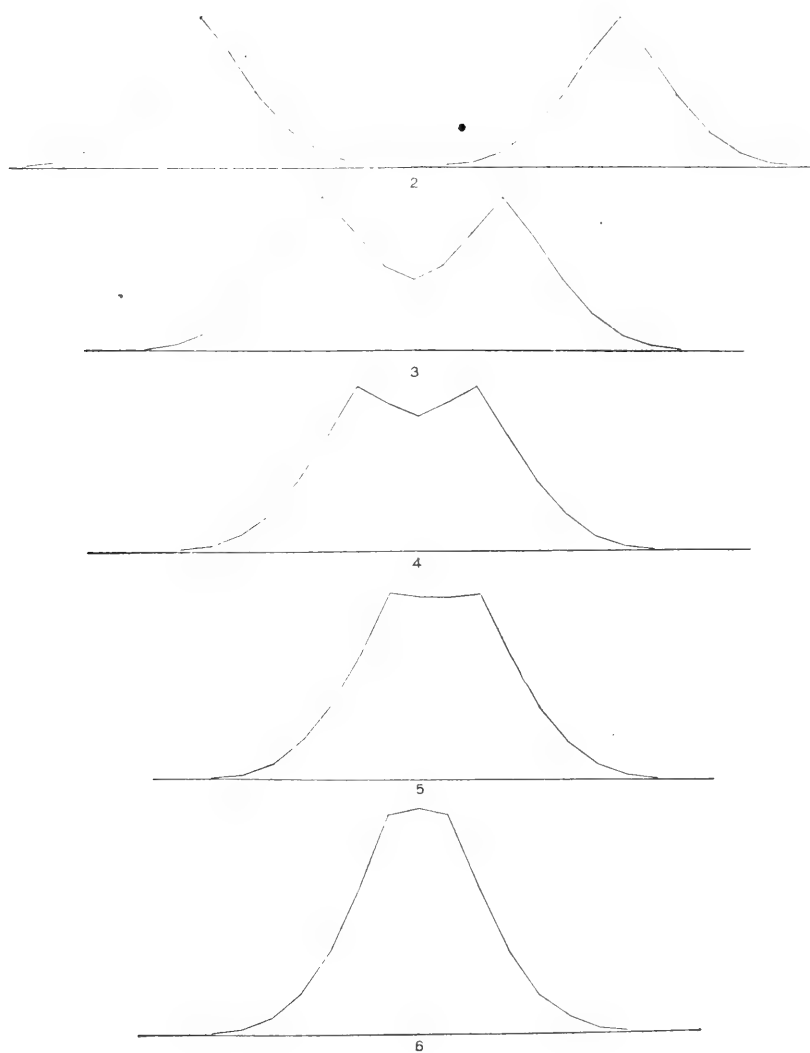
Component A	1	3	11	28	55	90	120	90	55	28	11	3	1				
Component B					1	3	11	28	55	90	120	90	55	28	11	3	1
Population	1	3	11	28	56	93	131	118	110	118	131	93	56	28	11	3	1

Fig. 5 A population of one thousand individuals consisting of two overlapping components of five hundred each, which are so close together as to produce a flat topped curve of distribution with just a trace of the two separate modes.

Component A	1	3	11	28	55	90	120	90	55	28	11	3	1				
Component B					1	3	11	28	55	90	120	90	55	28	11	3	1
Population	1	3	11	29	58	101	148	145	145	148	101	58	29	11	3	1	

Fig. 6 A population of one thousand individuals consisting of two overlapping components of five hundred each, which are so close together as to produce a unimodal curve of distribution.

Component A	1	3	11	28	55	90	120	90	55	28	11	3	1				
Component B					1	3	11	28	55	90	120	90	55	28	11	3	1
Population	1	3	12	31	66	118	175	180	175	118	66	31	12	3	1		



bution, are mixed, as according to hypothesis they are in the case of the spermatozoa in question, the distribution of individuals among the different sizes should differ from that found in a uniform population. It should furthermore be possible from the character of the distribution to determine something of the characters of the two component populations, the resultant depending among other things upon the degree of difference between the two components and upon the comparative numbers in the two.

Figure 1 is an ideal normal variability curve. Figures 2, 3, 4, 5 and 6 give the components and resultants of combinations between two populations of equal numbers. Various degrees of separation of the modes of the two populations are represented. In case the modes are widely separated the two populations come out as two entirely separate unimodal curves (fig. 2). In case they are closer together there are different degrees of overlapping giving resultant curves with two distinct modes (fig. 3), with less distinct modes (fig. 4), with one flattened mode (fig. 5) and finally with an appearance not to be distinguished by bare inspection from a curve of normal variability (fig. 6). These different appearances are due to different degrees of proximity of the component modes. In the last case given (fig. 6) where the components are close together, it is not possible to determine from the resultant curve whether or not it is a single simple curve or the combination of two, though no doubt this might be done by mathematical analysis (Pearson, '94; '14).

If one of the two components has a larger number of individuals than the other, and the modes are close enough to bring an overlapping of sizes the resultant curves may be represented by figures 7, 8 and 9. Different degrees of separation of the component modes are again represented.

It is evident from the above cases that there may be a question as to presence of two or only one component in a population which shows an approximation to the Gaussian curve in its group distribution because in case the modes of two component groups are close together the resultant may closely resemble a simple curve. On the other hand, if there is a pronounced de-

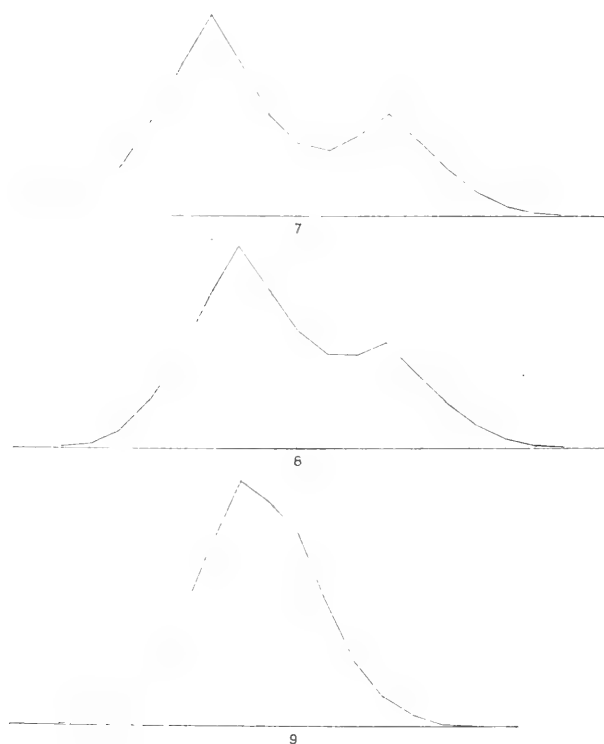


Fig. 7 A population of one thousand individuals consisting of two overlapping components, one twice as numerous as the other, and exhibiting two distinct modes when combined.

Component A	2	4	14	38	74	120	160	120	74	38	14	4	2							
Component B								1	2	7	19	37	60	80	60	37	19	7	2	1
Population	2	4	14	38	74	120	161	122	78	57	51	64	82	60	37	19	7	2	1	

Fig. 8 A population of one thousand individuals consisting of two overlapping components, one twice as numerous as the other, and exhibiting two modes when combined.

Component A	2	4	14	38	74	120	160	120	74	38	14	4	2							
Component B								1	2	7	19	37	60	80	60	37	19	7	2	1
Population	2	4	14	38	74	121	162	127	93	75	74	84	62	37	19	7	2	1		

Fig. 9 A population of one thousand individuals consisting of two components, one twice as numerous as the other, and exhibiting when combined an asymmetrical distribution with a single mode.

Component A	2	4	14	38	74	120	160	120	74	38	14	4	2						
Component B				1	2	7	19	37	60	80	60	37	19	7	2	1			
Population	2	4	15	40	81	139	197	180	154	98	51	23	9	2	1				

parture from the Gaussian form so that there is a marked flatness of the top of the curve or two distinct modes it is almost certain where a large number of individuals are measured that the population must consist of at least two principal distinct components. The chance in a sample including five hundred or more individuals that a uniform population will show a distinctly humped or a bimodal appearance is very slight indeed.

Since in the length determination considered in the present paper there is a range of variation greater than the expected difference between the two hypothetical classes, the method of procedure must be the one outlined above. The size distribution of the population is obtained, and from its character an attempt is made to determine whether there is any evidence of heterogeneity and if so to determine the probability that this heterogeneity is of the kind to be expected on the basis of known chromosomal history.

Because of the smallness of the expected difference it is of the utmost importance to consider carefully the possibility that dimorphism or monomorphism in the data may represent something other than the actual condition of the living functional spermatozoa. Lack of care may easily introduce a spurious dimorphism not at all present in the original material. The reverse is also true that certain methods of technique and observation may obscure dimorphism that does exist in the living ripe material. Such a discrepancy between final data and original material may be introduced in many ways. The problem in hand is the determination of the presence or absence of two size groups in a single testis. The possible sources of error must therefore be carefully considered.

6. Possible sources of error

Having determined the character of the variation curve it can not at once be concluded that the character of the living functional spermatozoa has been discovered. The curve is obtained from the measurements as put down in the note-book. There is (a) first of all the possibility that certain peculiarities

of the curve are due to the personal equation of the observer, or to other errors in measurement, and are not at all represented in the material on the slide. There is (b) the further possibility that the material on the slide does not represent the true character of the living material. The method of preparation may have introduced differences, giving the characteristic features to the curve. Finally (c) there is the question of the significance of the characteristics of the living sample if no errors are considered to have been introduced by measurement, or by other parts of the technique. For example, if two size groups actually exist in the living sample obtained they may have been produced by any one of a number of conditions quite apart from differences in chromatin content.

It remains to consider more specifically the possible sources of error under each of the heads mentioned, and the means pursued in attempting to eliminate them.

(a) *Sources of error connected with measurement.* (1) All spermatozoa which were not in perfect focus at both ends at one time were discarded because the fore-shortening would have introduced an error. It is possible that the longer spermatozoa are more frequently discarded for this reason than the shorter ones, because they may more frequently be in this tilted position. This would tend to introduce a spurious preponderance of the shorter ones. There is no reason to believe, however, that such an error would introduce a false bimodality. It might, however, bring asymmetry into a bimodal distribution.

(2) It took a number of sittings to complete a single set of measurements. The bimodality may be due to different standards of measurement used at the different times, resulting from change in intensity of light, in fatigue, etc. The precautions taken to avoid such an error are discussed above (pp. 190-191).

(3) The character of the scale used may bring about a tendency to aggregate individuals around certain points. Thus a slightly heavier and therefore thicker line at a certain point in the scale is very likely to acquire a wider zone than a narrow line and for this reason will have a greater number of individuals credited to it.

The observer's personal equation may lead him to prefer certain tenths to others, the wholes to the halves, or vice versa, and especially certain quarters to others. Numerous tests were made by using scales which differed from each other in the value of their units, and by using different observers for the same material. It will be noted that in most of the cases at least two measurements were made of the spermatozoa of a species, often by different observers.

(4) Many individual spermatozoa come near the border line between two of the smallest units of measurement. An observer who has obtained certain results in previous work, or who has a hypothesis as to what may be expected is likely unconsciously to influence the data thereby if he does not recognize the possibility. If he does recognize it, and is most scrupulous in attempting to avoid the introduction of such a bias he may get into error in the other direction. If he is over-zealous in the desire to be fair he may throw all doubtful cases in such a way as to obscure the truth.

(b) *Possible sources of error in connection with the preparation of the material for measurement.* (1) Differences in fixation or staining at different parts of the slide may cause apparent dimorphism in case there are two regions on the same slide which differ distinctly in this regard. In several of the species the data were tabulated separately for different regions of the slide, and dimorphism appeared in each of them. In these cases this source of error is eliminated.

(2) When the spermatozoa are spread out on the slide drying proceeds rapidly at the edges. This may cause a greater shrinking along the edges of the cover-glass than at the center. Tabulation from the center and from the sides of the cover-glass, however, showed no essential difference between the two regions.

(3) In some of the first slides examined aceto-carmin was used as a fixing agent and stain. The living spermatozoa were put under an oblong cover-glass and the aceto-carmin was drawn through by the action of filter paper placed at one end of the long axis. The measurements showed a difference in size of the spermatozoa at the two ends of the cover-glass. Such material

of course was discarded for present purposes but the case is interesting because it shows another way in which regional differences may be produced on a slide. It furthermore indicates a possible means of separating larger from smaller living spermatozoa in cases where it is desired to test them in fertilization experiments.

(4) In spreading seminal fluid on a slide coated with an albumen film individual spermatozoa are sometimes stretched and otherwise distorted. Such distorted individuals can often be readily recognized, but it is undoubtedly true that slightly stretched ones may be measured. Such stretched individuals may be introduced into the data and form a class of their own which may make up one of the dimorphic groups. To avoid such a contingency slides with many stretched spermatozoa were discarded.

(5) Some of the spermatozoa on practically all the slides are curved. In case the curvature is in the plane of the slide both ends may be in focus at the same time, and the curved as well as the straight spermatozoa may be measured. In case there is one definite degree of curvature which is so slight as to be often confused with the straight condition an apparent dimorphism may be introduced. To avoid this care was taken to measure only straight spermatozoa and to discard entirely slides with many curved individuals.

(6) In case the larger spermatozoa tend to curve more readily than the shorter ones, or vice versa, discarding of the curved individuals may introduce a spurious unimodal condition, or cause an asymmetrical bimodal curve in cases in which the normal population is symmetrically bimodal.

(7) The chromatin rod takes a heavy strain, and the cytoplasmic cap a light one. In some cases, especially in the short blunt spermatozoa of vertebrates, the cytoplasmic cap may make it difficult to get clearly the exact limit of the chromatin portion of the head. There may be a tendency to include the cytoplasmic band in certain of the cases and thereby produce an apparent dimorphism.

(c) *Possible erroneous interpretation of the character of the living sample considering the other sources of error to be eliminated.* (1)

The error of random sampling. It is possible that the size distribution obtained by taking a sample of the spermatozoa of a single testis is not the same as the one that would be obtained if all the spermatozoa of that testis were measured. The chance that a particular distribution may be the result of random sampling in any particular case can be determined by mathematical analysis. The number of individuals used in the present determination is generally considered to be sufficient to avoid any considerable error.

(2) If two or more different degrees of maturity are present in the sample studied an apparent dimorphism may appear. The precautions taken to avoid this are mentioned above (p. 189). In order that a distinct dimorphism due to this cause may appear it is necessary that growth during transformation of spermatid to spermatozoon be not uniform. There must be a very rapid change to the end product from a distinct stage which is at casual glance indistinguishable from the fully completed one. There will then be few intermediate forms at any particular time, but comparatively larger numbers of the earlier and later stages.

(3) There may be an accumulation of spermatozoa of a particular size at one time in a particular part of the efferent ducts of the testis because of differences in physiological activity, or possibly because of a mechanical separation. This would of course tend to obscure any dimorphism that might be present if we took the whole mass of spermatozoa of the testis. So far as this factor is concerned any dimorphism that has been made out would merely be accentuated if the error were corrected.

(4) The chromatin rod length of the sperm-head may differ from the total sperm-head length in frequency distribution. Since the chromatin rod length is so near the total length of the sperm-head this contingency need not be seriously considered.

(5) If spermatozoa from two different individuals or from the two testes of a single individual were mixed together the measurements of such a population might show a dimorphism not due

to differences present within a single testis. Therefore throughout this study only spermatozoa from single testes were measured in a single set.

While there are a very great many possible sources of error, and they should not be minimized in such determinations as the present, yet with the precautions taken, when the cumulative effect of the whole mass of evidence is considered, it is impossible that the conclusion to be drawn from the work as a whole can be in doubt. It is also to be noted that many of the possible sources of error tend to obscure rather than accentuate a probable dimorphism.

DATA AND RESULTS

1. *Musca domestica*

(a) *Spermatogenesis evidence concerning dimorphism.* According to the description of N. M. Stevens ('08) X and Y chromosomes are present. One-half of the spermatids receive the larger, or X chromosome, and the other half the smaller, or Y, chromosome. According to the drawings the other chromosomal pairs are made up of equal components. The total amount of chromatin received by the spermatids with an X chromosome should therefore be greater than that received by the spermatids with a Y chromosome. The sperm-head (fig. 10 e) is made up almost wholly of dense chromatin material. Assuming that the degree of condensation of the chromatin does not differ in the two cases, and there is no reason to suppose such a difference, the size of the sperm-heads ought to be directly proportional to the amount of chromatin received. Knowing the volumes of chromatin, it ought to be possible to figure out the expected lengths of the two kinds of sperm-heads.

Calculations made from the published figures must of necessity be crude since the exact shapes cannot be determined. They are, however, of considerable value as a rough check on the length measurements. Miss Stevens' plate 1, figure 3, representing a late prophase of the first division was used for the measurement of the chromosomes in this species. The values obtained are

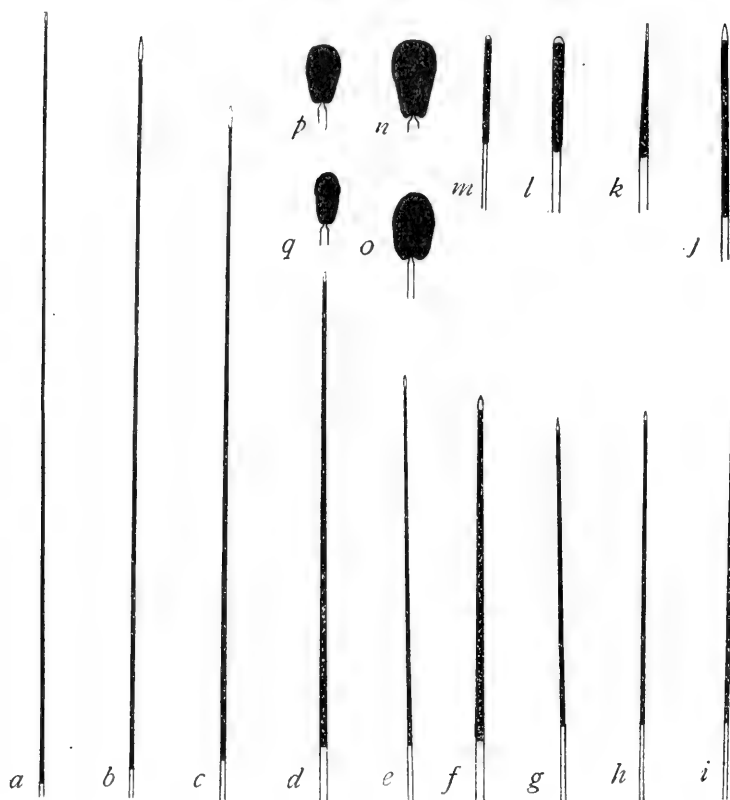


Fig. 10 Diagrams of heads of spermatozoa; camera lucida drawings. $\times 1340$.
a, *Melanoplus differentialis*; *b*, *Melanoplus femur-rubrum*; *c*, *Gryllus abbreviatus*;
d, *Aeshna canadensis*; *e*, *Musca domestica*; *f*, *Phytonomus punctatus*; *g*, *Lygaeus*
kalmii; *h*, *Anasa tristis*; *i*, *Alydus pilosulus*; *j*, *Trirhabda tomentosa*; *k*, *Psuedemys*
troostii; *l*, *Rana pipiens*; *m*, *Gallus domesticus*; *n*, *Bos taurus*; *o*, *Ovis aries*; *p*,
Canis familiaris; *q*, *Homo sapiens*.

not absolute lengths and breadths, as these are not necessary in order to obtain relative sizes:

Chromosomes	Width	Length	Chromosomes	Width	Length
a	3.6	12.0	e	3.5	17.0
b	2.9	15.0	Y	2.1	2.2
c	3.0	9.6	X	4.4	5.2
d	3.2	13.3			

One group of spermatids receives chromosomes a, b, c, d, e and Y, and the other group, chromosomes a, b, c, d, e, and X. Two determinations were made:

(1) The expected ratio between the lengths of the two classes of spermatozoa in case the chromatin fuses together to form one mass which is molded into a constant shape regardless of amount. In this case the resultant sperm-heads will have the same proportions between length, width and thickness regardless of size. The calculated ratio on this basis is length of sperm-heads with Y chromosome: length of sperm-heads with X chromosome:: 1.00 : 1.08.

(2) The expected ratio between the lengths of the two classes in case the chromosomes are united end to end to produce the chromatin rod of the spermatozoön. Since the figure gives the late prophase of the first maturation division the resultant length after end to end fusion of chromosomes depends upon the manner in which the material is divided during the divisions. If all divisions were strictly longitudinal, and there were no contraction, the resultant length would be the same as for the figure. In case the divisions bring about a reduction in length of each chromosome the maximum reduction that is possible is to one-fourth for the ordinary chromosomes and to one-half for X and Y chromosomes. Taking the two extreme possibilities the ratios are:

Sperm head with Y: sperm head with X :: 1.00 : 1.04

Sperm head with Y: sperm head with X :: 1.00 : 1.08

The former is true in case of longitudinal division of chromosomes, the latter in case of cross division of chromosomes. The first determination is probably nearer the truth than the second.

Combining the volume and end-to-end determinations the extreme ratios are 1.00 : 1.04 and 1.00 : 1.08. Leaving out the errors due to crudity of the measurement we may consider that somewhere between these values the true expected value is to be found. There is every reason to believe that the first determination (1), the one based on production of spermatozoa of

like shape, is the true one. The other determination is put in merely for the sake of completeness since we do not have absolute certainty on this point.

(b) *Material and method.* Obtained at Urbana, Illinois, May 18, 1913; exhibited motility in Ringer's solution; killed in osmic fumes; diagram of sperm-head in figure 10 e; measurements were made to halves of micrometer divisions, one division being equal to 1.1μ . Only about one in every fifty spermatozoa had to be discarded because of curvature. Only three spermatozoa in all three sets combined were found to be obviously immature. Other testes than those used in these measurements showed that motility is present in various stages before completion of the transformation of spermatids to spermatozoa.

(c) *Data.* Three series of measurements were made: (1) 444 spermatozoa from a right testis; the distribution of size groups is given in graphical and in numerical form in figure 11. The distribution is distinctly bimodal. (2) 465 spermatozoa from the left testis of the same individual (fig. 12). The distribution is again distinctly bimodal. (3) 784 spermatozoa from the same testis as (2) (fig. 13). The distribution is a third time distinctly bimodal.

(d) *Conclusion.* There is an evident bimodality in each of the three determinations. The general significance of such an observed bimodality has been discussed under the general section on "Materials and methods," especially on pages 189 to 201.

Here a special comparison may be made between the lengths as observed and as calculated on the basis of spermatogenesis descriptions and figures. If the bimodal curves represent a mixture of two populations each with a normal variation distribution, the distance between the two modes of the observed distribution is equal to or less than the distance between the modes of the two component curves (figs. 1 to 9). The observed modes for *Musca* are to each other as:

35.8 : 38.6 or 1.00 : 1.08 for figure 11

35.8 : 38.6 or 1.00 : 1.08 for figure 12

36.4 : 38.0 or 1.00 : 1.04 for figure 13

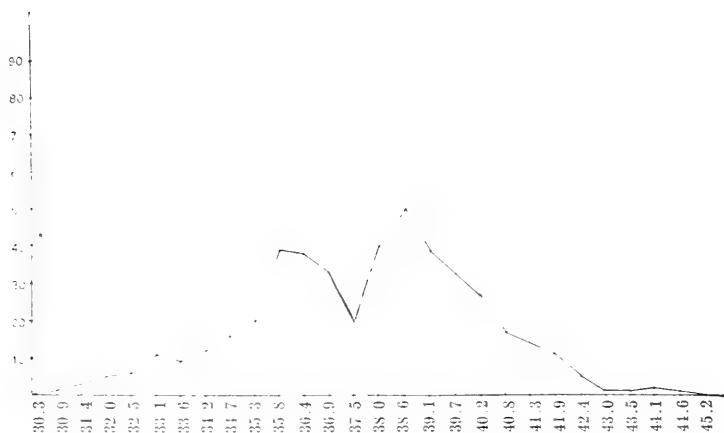


Fig. 11 *Musca domestica*: frequency distribution of head-lengths of 444 spermatozoa from right testis.

Value in μ	30.9	31.4	32.0	32.5	33.1	33.6	34.2	34.7	35.3	35.8
Frequency	1	3	5	6	11	9	12	16	20	39
	36.4	36.9	37.5	38.0	38.6	39.1	39.7	40.2	40.8	41.3
	38	33	20	40	50	39	33	27	17	14
	41.9	42.4	43.0	43.5	44.1	44.6				
	11	5	1	1	2	1				

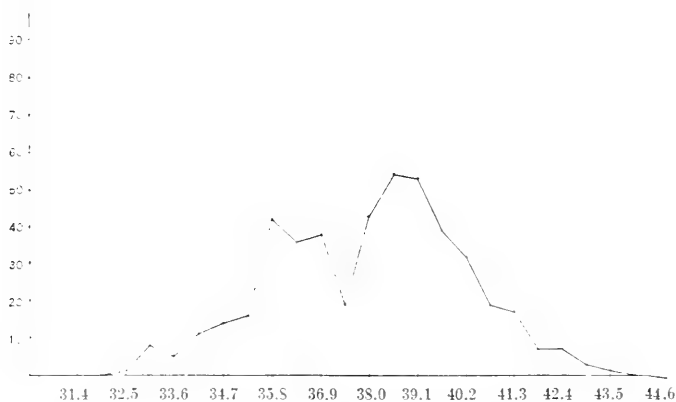


Fig. 12 *Musca domestica*: frequency distribution of head-lengths of 465 spermatozoa from left testis.

Value in μ	31.4	32.0	32.5	33.1	33.6	34.2	34.7	35.3	35.8	36.4
Frequency	0	0	1	8	5	11	14	16	42	36
	36.9	37.5	38.0	38.6	39.1	39.7	40.2	40.8	41.3	41.9
	38	19	43	54	53	39	32	19	17	7
	42.4	43.0	43.5	44.1						
	7	3	1	0						

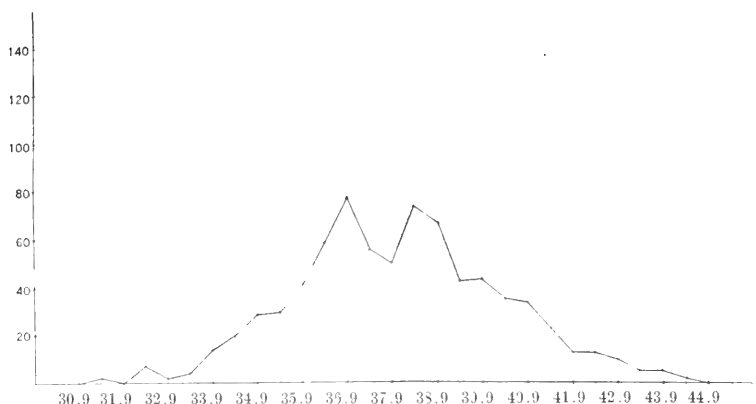


Fig. 13 *Musca domestica*; frequency distribution of head-lengths of 769 spermatozoa from same testis as the one in figure 12.

Value in μ	31.4	31.9	32.4	32.9	33.4	33.9	34.4	34.9	35.4	35.9
Frequency	2	0	7	2	4	14	20	29	50	43
	36.4	36.9	37.4	37.9	38.4	38.9	39.4	39.9	40.4	40.9
	60	79	57	51	75	68	43	44	36	34
	41.4	41.9	42.4	42.9	43.4	43.9	44.4			
	23	13	13	10	5	5	2			

These agree well with the values expected from the spermatogenesis determinations, which are between 1.00 : 1.04 and 1.00 : 1.08. This agreement strengthens the view that the two classes indicated by the bimodality of the variation curve are actually related to difference in chromatin content as observed in spermatogenesis.

There is an asymmetry of distribution in all three sets. The group with the larger individuals is slightly greater than the group with the smaller individuals. The significance of this asymmetry is uncertain. It may be due merely to one of the uncontrolled factors already mentioned under possible sources of error. The same may be said of the various minor irregularities in this and other determinations. If they are not due to errors creeping in during the course of preparation and observation they indicate that size of spermatozoa is influenced by several distinct factors.

2. *Lygaeus kalmii*

(a) *Spermatogenesis evidence concerning dimorphism.* While there is no description of this particular species, E. B. Wilson ('05 a) has described X and Y chromosomes in the related species *L. turcicus*. The measurements as taken from a side view of the metaphase of the second division (Wilson's fig. 11) give the following dimensions:

Chromosomes	Width	Length	Chromosomes	Width	Length
a	2.9	3.2	e	3.2	3.4
b	2.9	3.6	f	2.8	2.9
c	3.1	3.7	Y	0.9	1.9
d	3.3	3.6	X	2.0	3.2

One group of spermatids receives chromosomes a, b, c, d, e, f and Y, and the other group receives a, b, c, d, e, f and X. This gives a ratio for expected lengths of 1.00 : 1.02 on the basis of fusion of chromosomes to form spermatozoa of like shape or 1.00 : 1.06 on the basis of end to end fusion of chromosomes.

(b) *Material and method.* Obtained at Woods Hole, Massachusetts, September 1, 1913; exhibited motility; killed in osmic fumes; diagram of sperm-head in figure 10 g; measurement to halves of units of micrometer scale, one unit being equal to 0.996μ , in the first set, and 0.97μ in the second set. Only two curved individuals were found in the first set, and none in the second.

(c) *Data.* Two sets of measurements were made: (1) 493 spermatozoa from a right testis (fig. 14). The distribution is bimodal and indicates two groups in the population. (2) 501 spermatozoa from the same testis (fig. 15). The distribution is again bimodal.

(d) *Conclusion.* The spermatozoa do not constitute a uniform population. There are two distinct size groups. The observed modes are to each other as

$$\begin{aligned} &36.8 : 38.3 \text{ or } 1.00 : 1.04 \text{ in the first set} \\ &36.8 : 37.8 \text{ or } 1.00 : 1.03 \text{ in the second set} \end{aligned}$$

These values come between the 1.00 : 1.02 and the 1.00 : 1.06 ratios calculated from the chromosomal differences of the closely

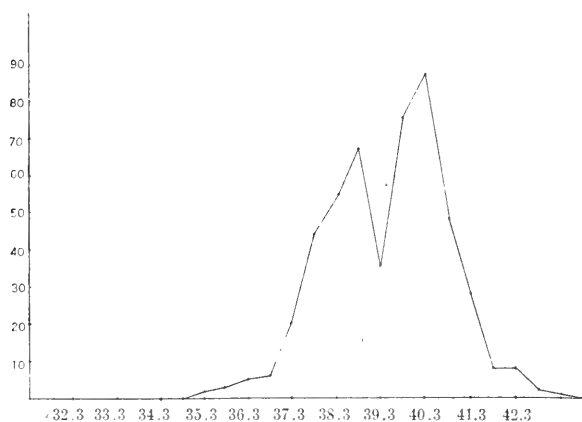


Fig. 14 *Lygaeus kalmii*; frequency distribution of head-lengths of 493 spermatozoa from the right testis.

Value in μ	33.3	33.8	34.3	34.8	35.3	35.8	36.3	36.8	37.3	37.8
Frequency	2	3	5	6	20	44	54	67	35	75
	38.3	38.8	39.3	39.8	40.3	40.8	41.3			
	87	48	28	8	8	2	1			

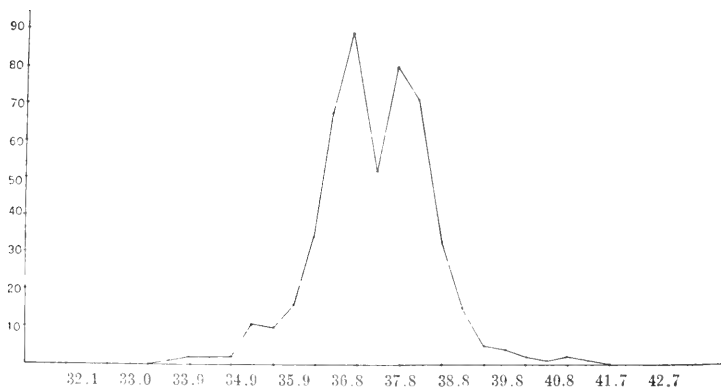


Fig. 15 *Lygaeus kalmii*; frequency distribution of head-lengths of 501 spermatozoa from the same testis as the one in figure 14.

Value in μ	32.5	33.0	33.5	33.9	34.4	34.9	35.4	35.9	36.3	36.8	
Frequency	1	2	2	2	11	10	16	34	68	89	
	37.3	37.8	38.3	38.8	39.2	39.7	40.2	40.7	41.2	41.6	42.1
	52	80	71	33	15	5	4	2	1	2	1

related species *L. turcicus*. There is a high degree of probability, therefore, that the two size groups are correlated with chromosomal differences.

3. *Alydus pilosulus*

(a) *Spermatogenesis evidence concerning dimorphism.* According to the description of E. B. Wilson ('05 b) there is an accessory or X chromosome. One-half of the spermatids ought therefore to differ in chromatin content from the other half by the value of this chromosome. The measurements of the chromosomes as taken from Wilson's figure e of the late prophase of the first division are the following:

Chromosomes	Width	Length	Chromosomes	Width	Length
a	3.4	3.9	e	3.2	3.4
b	4.6	4.9	f	3.2	4.9
c	4.4	5.0	X	3.9	4.8
d	1.8	3.4			

One-half of the spermatids receive chromosomes a, b, c, d, e and f, and the other half a, b, c, d, e, f and X. Using the method described under *Musca* (p. 201) the expected ratios of length of sperm-heads without X to length of sperm-heads with X are 1.00 : 1.06 for complete fusion and remolding, and 1.00 : 1.09 to 1.19 for end to end fusion of chromosomes.

(b) *Material and method.* Obtained at Urbana, Illinois, October 15, 1913; the spermatozoa all mature, the individual being one left over from the summer and not a young animal; killed in osmic fumes; diagram of a spermatozoön shown in figure 10 i; measurements to whole units in the first set and to quarter units in the second though the latter are grouped in halves because of internal evidence of a tendency to neglect the quarters. One unit of micrometer equals 0.996μ .

(c) *Data.* Two sets of measurements were made:

(1) 429 spermatozoa from a right testis (fig. 16). The distribution is bimodal. (2) 469 spermatozoa from the same right testis (fig. 17). There is a bimodality with marked asymmetry.

(d) *Conclusion.* The spermatozoa do not constitute a uniform population. There is distinct evidence of two main groups.

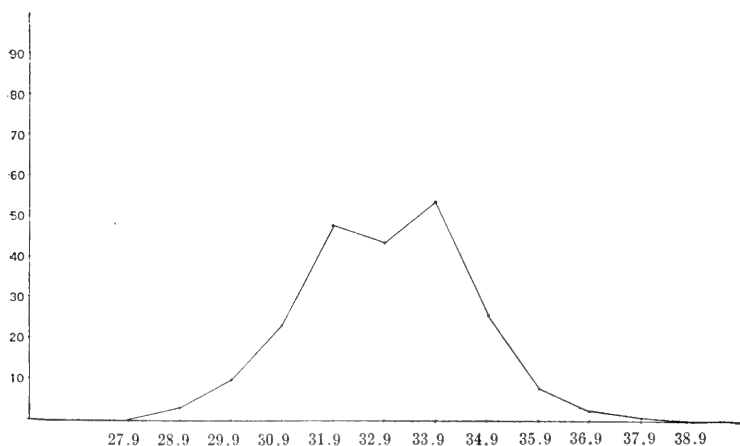


Fig. 16 *Alydus pilosulus*; frequency distribution of head-lengths of 429 spermatozoa from right testis.

Value in μ	28.9	29.9	30.9	31.9	32.9	33.9	34.9	35.9	36.9
Frequency	6	19	46	94	86	106	51	16	5

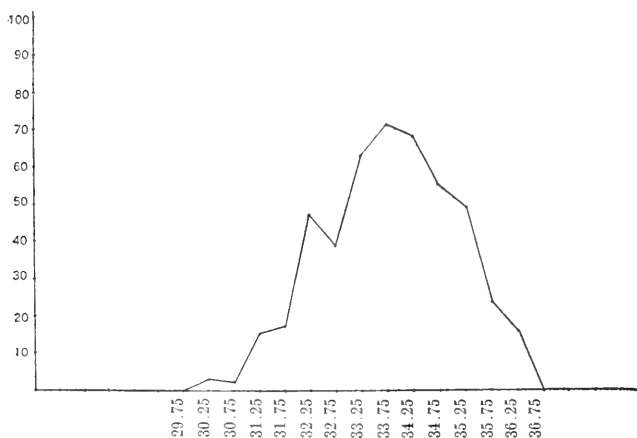


Fig. 17 *Alydus pilosulus*; frequency distribution of head-lengths of 469 spermatozoa from the same testis as the one in figure 16.

Value in μ	30.2	30.7	31.2	31.7	32.2	32.7	33.2	33.7	34.2	34.7
Frequency	3	2	15	17	47	39	63	71	68	55
	35.2	35.7	36.2							
	49	24	16							

The modes for the first set are 31.9μ and 33.9μ giving a ratio of 1.00 : 1.06. The modes for the second set are 32.25μ and 33.75μ giving a ratio of 1.00 : 1.05. This is very close to the ratio, 1.00 : 1.06, expected on the basis of complete fusion of chromosomes and formation of spermatozoa all of which are of the same shape.

4. *Anasa tristis*

Size dimorphism in the spermatozoa has been described by the junior author (Faust '13). The following account gives some new measurements in addition to the old ones, and also adds the calculations of the length ratio to be expected on the basis of chromosomal history.

(a) *Spermatogenesis evidence concerning dimorphism.* An X chromosome has been described by E. B. Wilson ('05 b) and others. The dimensions of the chromosomes for the metaphase of the first division as given in Wilson's figure 20 are as follows:

Chromosomes	Width	Length	Chromosomes	Width	Length
a	3.2	8.6	f	3.5	5.2
b	3.8	6.1	g	3.3	6.6
c	3.5	4.8	h	3.0	3.9
d	3.2	5.7	i	1.5	1.5
e	3.3	5.0	X	2.9	10.2

One-half of the spermatids receive chromosomes a, b, c, d, e, f, g, h and i, and the other half the same with the addition of X. Using the methods applied above to *Musca* (p. 201) the following ratios are obtained between sperm-head length without the X chromosome and sperm-head length with the X chromosome. In case there is complete fusion and remodeling to uniform proportions, 1.00 : 1.11, in case of end to end fusion 1.00 : 1.21 or 1.00 : 1.42 according to behavior of chromosomes during the maturation divisions.

(b) *Material and method.* A diagram of the sperm-head is given in figure 10 h. The new sets of measurements do not involve any new method; (see E. C. Faust '13).

(c) *Data.* Ten sets of measurements have been made. These are given in figures 18 to 27.

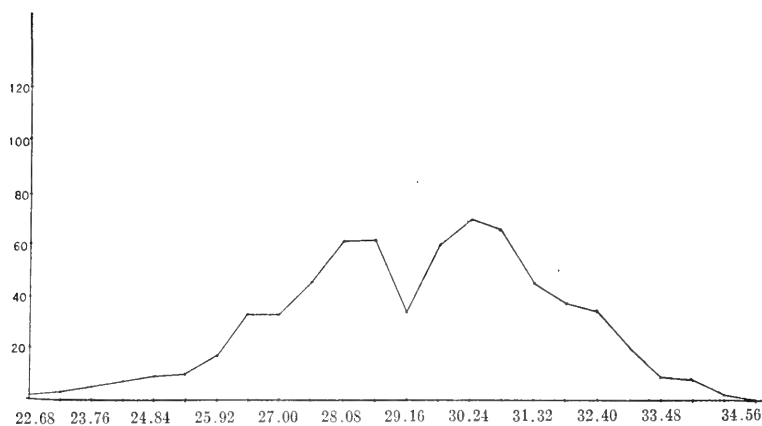


Fig. 18 *Anasa tristis*; frequency distribution of head-lengths of 653 spermatozoa from right testis.

Value in μ	22.7	23.2	23.8	24.3	24.8	25.4	25.9	26.5	27.0	27.5
Frequency	2	3	5	7	9	10	17	33	33	45
	28.1	28.6	29.2	29.7	30.2	30.8	31.3	31.8	32.4	32.9
	61	61	34	59	69	65	45	37	34	20
										9
										8
										2

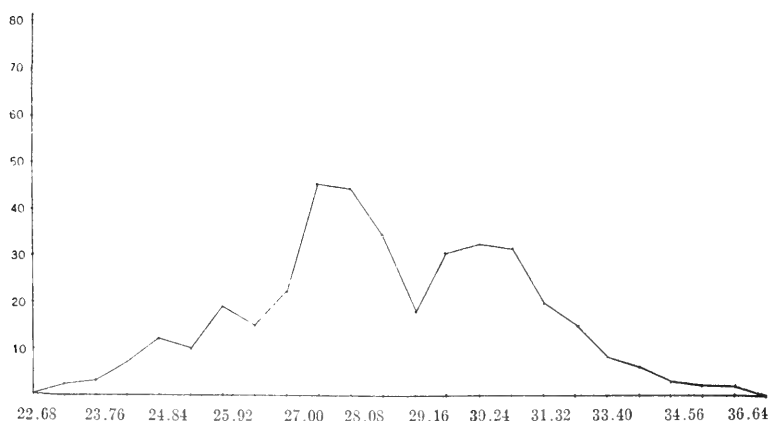


Fig. 19 *Anasa tristis*; frequency distribution of head-lengths of 391 spermatozoa from left testis of same individual as figure 18.

Value in μ	22.7	23.2	23.8	24.3	24.8	25.4	25.9	26.5	27.0	27.5
Frequency	0	2	3	7	12	10	19	15	22	45
	28.1	28.6	29.2	29.7	30.2	30.8	31.3	31.8	32.4	32.9
	44	34	18	30	32	31	20	15	9	6
	33.5	34.0	34.6							
	3	2	2							

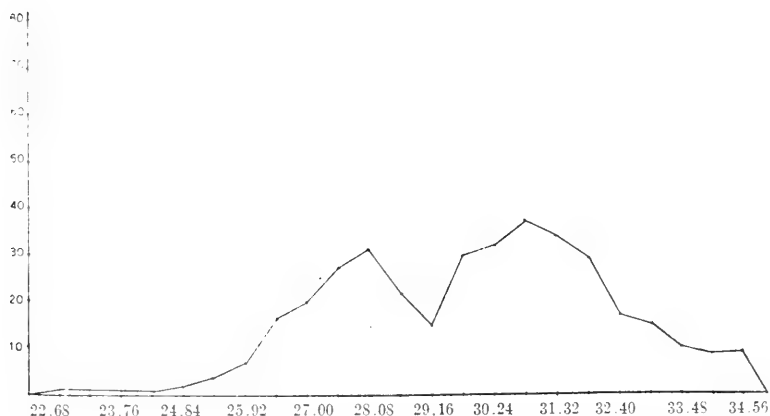


Fig. 20 *Anasa tristis*; frequency distribution of head-lengths of 370 spermatozoa from right testis.

Value in μ	22.7	23.2	23.8	24.3	24.8	25.4	25.9	26.5	27.0	27.5
Frequency	1	1	1	1	2	4	7	16	20	27
	28.1	28.6	29.2	29.7	30.2	30.8	31.3	31.8	32.4	32.9
	31	22	15	30	33	37	34	29	17	15
	33.5	34.0	34.6							
	10	9	9							

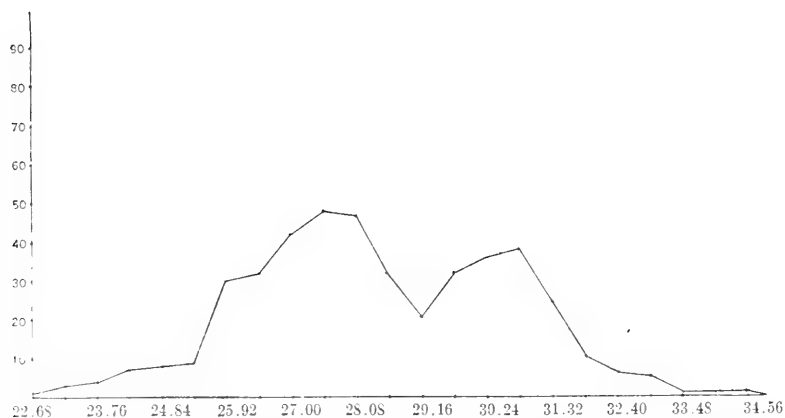


Fig. 21 *Anasa tristis*; frequency distribution of head-lengths of 443 spermatozoa from left testis of same individual as the one in figure 20.

Value in μ	22.7	23.2	23.8	24.3	24.8	25.4	25.9	26.5	27.0	27.5
Frequency	1	3	4	7	8	9	30	32	42	48
	28.1	28.6	29.2	29.7	30.2	30.8	31.3	31.8	32.4	32.9
	47	32	21	32	36	38	25	14	6	5
	33.5	34.0	34.6							
	1	1	1							

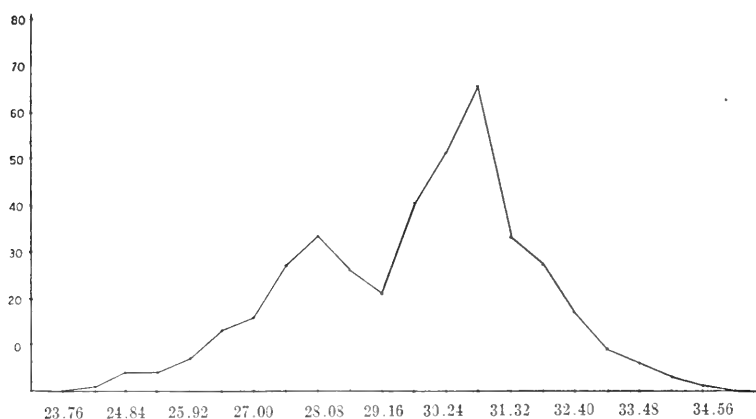


Fig. 22 *Anasa tristis*; frequency distribution of head-lengths of 404 spermatozoa from right testis.

Value in μ	24.3	24.8	25.4	25.9	26.5	27.0	27.5	28.1	28.6	29.2
Frequency	1	4	4	7	13	16	27	33	26	21
	29.7	30.2	30.8	31.3	31.8	32.4	32.9	33.5	34.0	34.6
	40	51	65	33	27	17	9	6	5	1

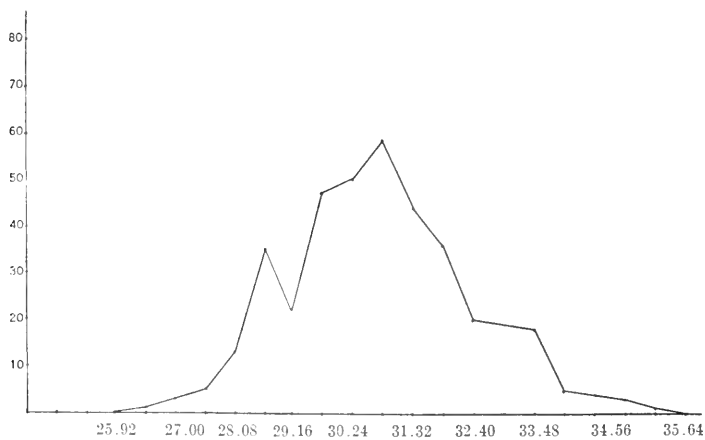


Fig. 23 *Anasa tristis*; frequency distribution of head-lengths of 384 spermatozoa from left testis of same individual as figure 22.

Value in μ	26.5	27.0	27.5	28.1	28.6	29.2	29.7	30.2	30.8	31.3
Frequency	1	3	5	13	35	22	47	50	58	44
	31.8	32.4	32.9	33.5	34.0	34.6	35.1	35.6		
	36	20	19	18	5	4	5	1		

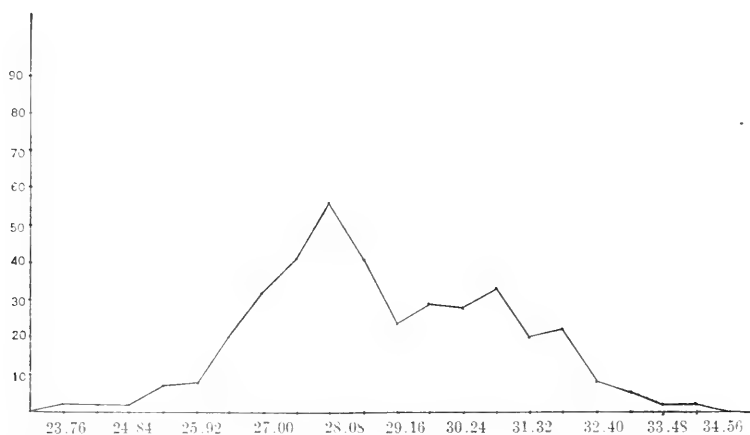


Fig. 24 *Anasa tristis*; frequency distribution of head-lengths of 394 spermatozoa from right testis.

Value in μ	25.8	24.3	24.8	25.4	25.9	26.5	27.0	27.5	28.1	28.6	29.2
Frequency	2	2	2	7	8	20	32	41	56	41	24
	29.7	30.2	30.8	31.3	31.8	32.4	32.9	33.5	34.0	34.6	
	20	28	33	20	22	8	5	2	2	0	

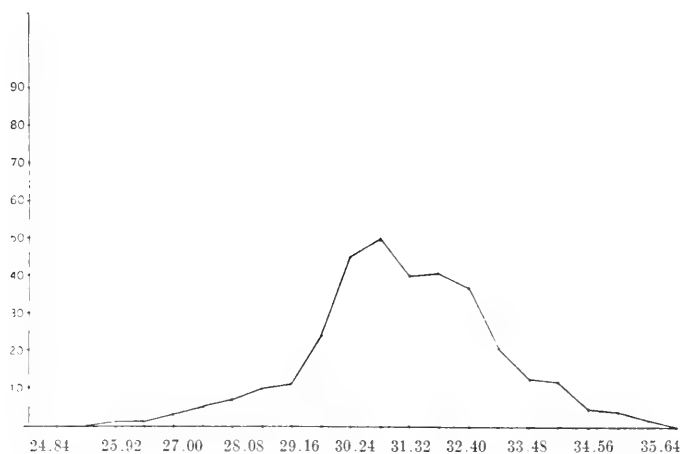


Fig. 25 *Anasa tristis*; frequency distribution of head-lengths of 322 spermatozoa from left testis of same individual as the one in figure 24.

Value in μ	25.9	26.5	27.0	27.5	28.1	28.6	29.2	29.7	30.2	30.8	31.3
Frequency	1	1	3	5	7	10	11	24	45	50	40
	31.8	32.4	32.9	33.5	34.0	34.6	35.1	35.6			
	41	37	21	13	12	5	4	2			

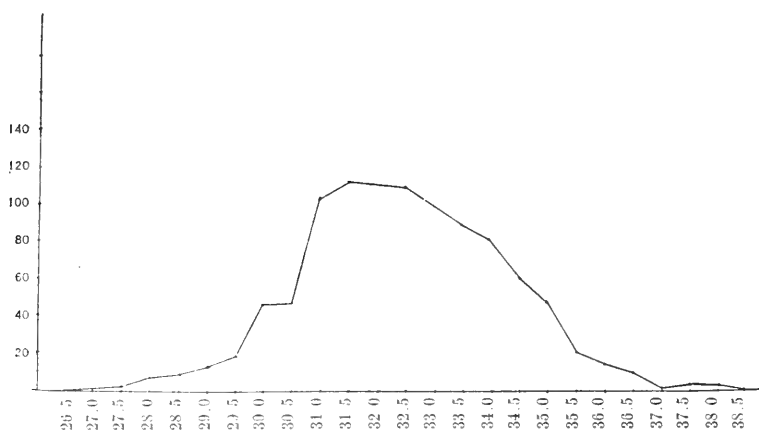


Fig. 26 *Anasa tristis*; frequency distribution of head-lengths of 993 spermatozoa from same left testis as the one in figure 25.

Value in μ	27.0	27.5	28.0	28.5	29.0	29.5	30.0	30.5	31.0	31.5
Frequency	1	2	7	9	13	19	47	48	103	112
	32.0	32.5	33.0	33.5	34.0	34.5	35.0	35.5	36.0	36.5
	111	109	99	89	81	61	47	21	15	10
	37.0	37.5	38.0							
	2	4	3							

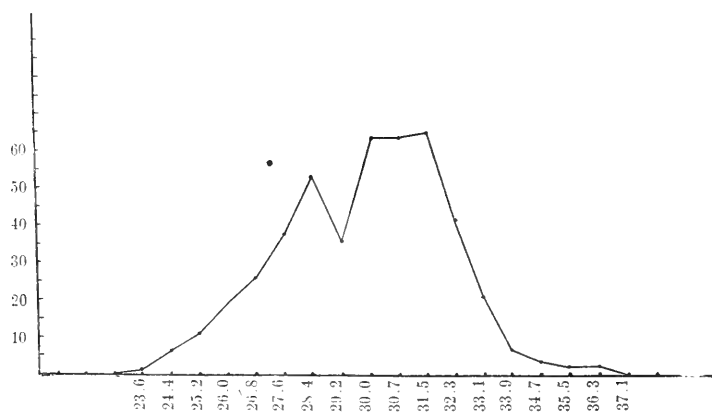


Fig. 27 *Anasa tristis*; frequency distribution of head-lengths of 444 spermatozoa from left testis.

Value in μ	23.6	24.4	25.2	26.0	26.8	27.6	28.4	29.2	30.0	30.7
Frequency	1	6	11	19	26	37	53	36	63	63
	31.5	32.3	33.1	33.9	34.7	35.5	36.3	37.1	37.9	38.7
	64	41	20	6	3	2	2	0	0	1

(d) *Conclusion.* Nine of the ten sets of measurements show very distinctly a bimodal distribution. The one unimodal set agrees very closely with the upper group of a bimodal set. Considering the evidence as a whole there is no escape from the conclusion that there are two principal size groups among the spermatozoa of *Anasa tristis*. The high points in nearly all the cases come at essentially the same places in the different measurements, though there is considerable variation in details. That the two size groups are dependent upon chromosomal differences is made very probable by a comparison of the observed and expected ratios. The two modes and the ratios for the different sets are as follows:

<i>Set</i>	<i>Figure</i>	<i>Lower mode</i>	<i>Upper mode</i>	<i>Ratio</i>
A	18	28.35	30.2	1.00 : 1.07
B	19	27.5	30.2	1.00 : 1.10
C	20	28.1	30.8	1.00 : 1.10
D	21	27.5	30.8	1.00 : 1.12
E	22	28.1	30.8	1.00 : 1.10
F	23	28.6	30.8	1.00 : 1.08
G	24	28.1	30.8	1.00 : 1.10
{H	25	30.81	31.8	1.00 : 1.03
{I	26	none	31.5	
J	27	28.4	31.5	1.00 : 1.11

With the exception of H and I, both of which are from the same testis, the observed ratios are very close to the ratio 1.00 : 1.11 as calculated from the chromosomal history on the assumption that the chromosomes fuse into one mass which is molded to form a sperm-head always of constant shape regardless of size. H shows a slight indication of bimodality which does not correspond with that of the others. I is unimodal with the mode agreeing closely with the upper mode of the bimodal sets. The only probable explanation of this unimodal condition is that one of the two groups of spermatozoa has disappeared. Since the single mode is very close to the upper mode of the bimodal cases the lost group would have to be the one with the smaller individuals, thus agreeing in certain respects with the permanent condition described for *Phylloxera* by T. H. Morgan ('09).

The asymmetry in the curves indicates that the two components of the population are not equal. Since according to the spermatogenesis facts the individuals of the two classes of spermatozoa should be produced in equal numbers it is necessary to assume that the sample chosen for measurement is not a fair sample of the whole population (see possible sources of error, paragraph 3, p. 200) or that more individuals degenerate in one group than in the other. If the physiological characteristics of the two groups differ there should be a tendency for the spermatozoa at any particular place, such as that from which the sample is taken, to be preponderately of one group. In fact this asymmetry strengthens the hope that it may be possible to devise some means of isolating the living spermatozoa of the two groups for experimental purposes. There seems, however, to be no satisfactory explanation of the reciprocal relation between the distributions in figures 17 and 18. The larger individuals are the more numerous in set A taken from a right testis, while in set B taken from the left testis of the same individual the smaller ones are the more numerous. A similar relation holds for figures 19 and 20. This seems to be a chance relation though it may have some interest to those who hold that the right and left testes normally differ in sex determining factors.

While the method is not accurate enough to enable one to make sure of the details there seems to be no doubt of the main fact of existence of two size groups agreeing closely with expectation on the basis of chromosomal history.

5. *Trirhabda tomentosa*

(a) *Spermatogenesis evidence concerning dimorphism.* There is no published description of the chromosomal relations in *T. tomentosa*, but Miss Stevens ('06) has described X and Y chromosomes for the related species *T. virgata*. The dimensions given below are taken from her plate 8, figure 16, which shows the first spermatocyte daughter plates:

<i>Chromosomes</i>	<i>Width</i>	<i>Length</i>	<i>Chromosomes</i>	<i>Width</i>	<i>Length</i>
a	1.8	2.0	i	1.4	1.8
b	1.3	1.9	j	1.3	2.0
c	1.1	1.5	k	1.3	1.6
d	1.3	1.8	l	1.6	1.9
e	1.5	2.0	m	1.3	1.7
f	1.6	1.6	Y	0.6	0.6
g	1.6	1.7	X	1.5	2.0
h	1.2	1.8			

These data give the following expected ratios between the head-lengths of the two kinds of spermatozoa: (1) In case of complete fusion into one mass and moulding into spermatozoa always of the same shape, 1.00 : 1.06. (2) In case of end to end fusion of chromosomes the same ratio, 1.00 : 1.06.

(b) *Material and method.* Collected at Douglas Lake, Michigan, July 30, 1913; motile in Ringer's solution; killed in osmic fumes; diagram of a sperm-head in figure 10 j, measurements are to tenths of micrometer divisions, one division being equal to 0.948 micron. A considerable number of the spermatozoa in the preparation are curved. These were discarded during measurement.

(c) *Data.* The 481 lengths measured are shown in figure 28. The distribution is distinctly bimodal, though with the modes close together so as to give almost a flat appearance of the type shown in figures 4 or 5. The two modes are at 17.02μ and 17.78μ giving a ratio of 1.00 : 1.045. This is not far from the expected ratio of 1.00 : 1.06.

(d) *Conclusion.* There is strong evidence of the existence of two principal size groups of spermatozoa probably based on chromosomal differences.

6. *Phytonomus punctatus*

(a) *Spermatogenesis evidence concerning dimorphism.* The chromosomal history of this species is not known, but since many beetles have been shown to have either X or X and Y chromosomes two size groups are to be expected in this case.

(b) *Material and method.* Material collected at Urbana, Illinois, November 17, 1913; killed in Zenker's fluid; diagram of

a sperm-head in figure 10 f; measurements are to halves of micrometer divisions, one division being equal to 0.996μ in the first set, and 0.97μ in the second set.

(c) *Data.* Set A, figure 29; 506 spermatozoa from the left testis. The distribution is bimodal with modes at 33.0μ and

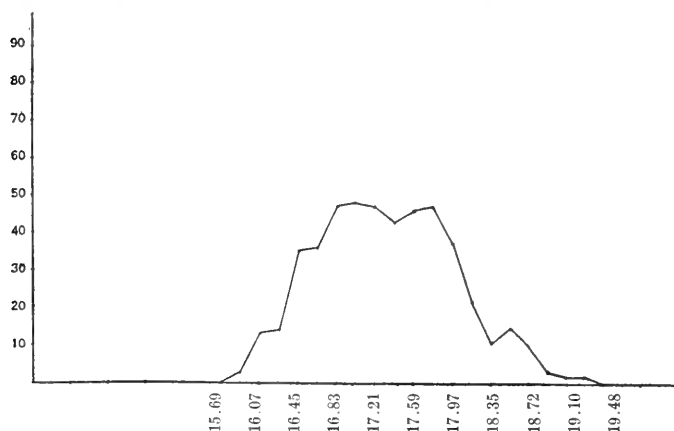


Fig. 28 *Trirhabda tomentosa*; frequency distribution of head-lengths of 481 spermatozoa from a single testis.

Value in μ	15.88	16.07	16.26	16.95	16.64	16.83	17.02	17.21	17.40
Frequency	3	13	14	35	36	47	48	47	43
	17.59	17.78	17.97	18.16	18.35	18.53	18.72	18.91	19.09
	46	47	37	22	11	15	10	3	2
	19.28								
	2								

34.0μ giving a ratio of 1.00 : 1.03. Set B, figure 30; 507 spermatozoa from the same left testis as figure 29. The distribution is bimodal with modes at 33.0μ and 35.3μ giving a ratio of 1.00 : 1.06.

(d) *Conclusion.* The two series of measurements do not agree closely, but both show distinctly that the population is not a uniform one and that two principal groups are involved.

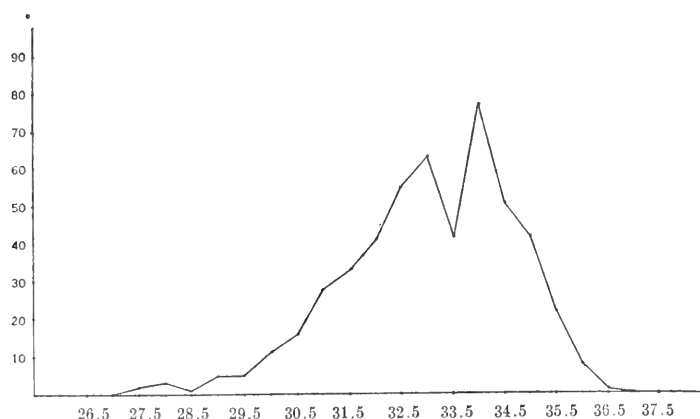


Fig. 29 *Pythonomus punctatus*; frequency distribution of head-lengths of 506 spermatozoa from left testis.

Value in μ	27.5	28.0	28.5	29.0	29.5	30.0	30.5	31.0	31.5	32.0
Frequency	2	3	1	5	5	11	16	28	33	41
	32.5	33.0	33.5	34.0	34.5	35.0	35.5	36.0	36.5	
	55	63	42	77	51	42	22	8	1	

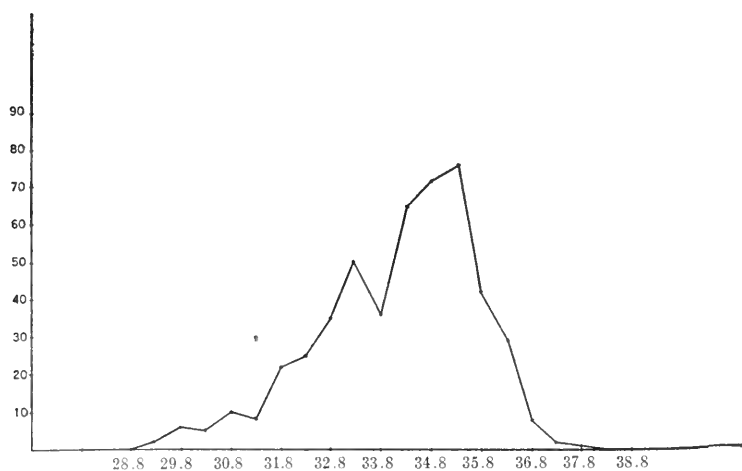


Fig. 30 *Pythonomus punctatus*; frequency distribution of head-lengths of 507 spermatozoa from the same left testis as the one in figure 29.

Value in μ	29.3	29.8	30.3	30.8	31.3	31.8	32.3	32.8	33.3	33.8
Frequency	2	6	5	10	13	22	25	35	50	36
	34.3	34.8	35.3	35.8	36.3	36.8	37.3	37.8		
	65	71	75	42	29	8	2	1		

7. Melanoplus femur-rubrum

(a) *Spermatogenesis evidence concerning dimorphism.* An accessory chromosome has been shown for this species from the description and figures of E. V. Wilcox ('95, '96) by C. E. McClung and others. Miss Carothers ('13) has recently shown the existence of an unequal pair of chromosomes apart from the X chromosome in three Orthopterans. This pair has been shown to be independent of the X chromosome and there should be four kinds of spermatids as regards amount of chromatin received. Calling the larger member of the unequal pair 'A' and the smaller one 'a', these four kinds may be represented as follows: (1) Those with $XA\ n$; (2) those with $Xa\ n$; (3) those with $-A\ n$; (4) those with $-a\ n$. Whether such a relation is to be expected in the present species is of course conjectural.

(b) *Material and method.* Obtained at Urbana, Illinois, November 17, 1913; motile; killed in osmic fumes; diagram of sperm-head in figure 10 b; measurements to halves of micrometer divisions, one division being equal to 1.66μ .

(c) *Data.* Figure 31; 491 spermatozoa from a single testis. The distribution shows that the population is made up of several groups. There are two principal modes, 80.5μ and 83.0μ giving a ratio of 1.00 : 1.03. At the sides of the principal modes are minor modes, two of which, one on each side, seem to indicate important factors. May these have some relation to probable inequality in a non-sex determining pair of chromosomes?

(d) *Conclusion.* There seems to be no question as to the main fact that the population is not a uniform one, and that the distribution indicates two main size groups.

8. Melanoplus differentialis

(a) *Spermatogenesis evidence* for this species is not available, but other species of the same genus have an X chromosome, and in a nearly related form inequality in another pair of chromosomes has been described.

(b) *Material and method.* Obtained at Urbana, Illinois, October 6, 1913; motile; killed in osmic fumes; diagram of sperm-

head in figure 10 a; measurements to halves of a micrometer division, one division being equal to 1.7μ in sets A and B, and 1.6μ in set C.

(c) *Data*. Three sets of measurements were made: Set A, figure 32; 481 spermatozoa from a left testis. The principal modes are at 88.0μ and 90.5μ , giving a ratio of 1.00 : 1.03. Set

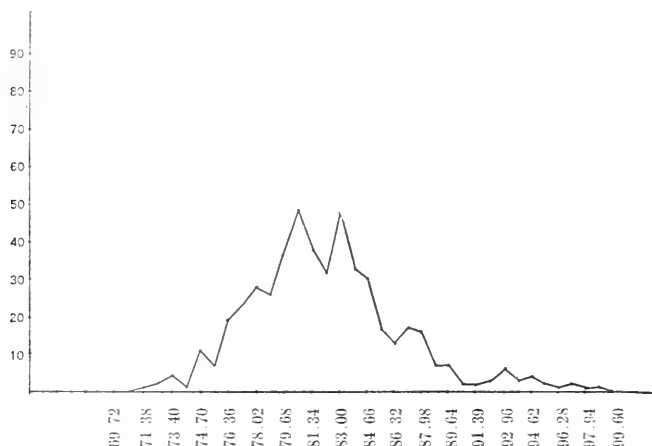


Fig. 31 *Melanoplus femur-rubrum*; frequency distribution of head-lengths of 491 spermatozoa from a single testis.

Value in μ	71.4	72.2	73.0	73.9	74.7	75.5	76.4	77.2	78.0	78.8
Frequency	1	2	4	1	11	7	19	23	28	26
	79.7	80.5	81.3	82.2	83.0	83.8	84.7	85.5	86.3	87.2
	37	48	38	32	48	33	30	17	13	17
	88.0	88.8	89.6	90.5	91.4	92.2	93.0	93.8	94.6	95.5
	16	7	7	2	2	3	6	3	4	2
	96.3	97.1	97.9							
	1	2	1							

B, figure 33; 1008 spermatozoa from the same left testis as figure 32. The principal modes are at 88.8μ and 90.5μ , giving a ratio of 1.00 : 1.02. Set C, figure 34; 734 spermatozoa from the same left testis as figures 32 and 33. The principal modes are at 88.5μ and 90.9μ , giving a ratio of 1.00 : 1.03.

(d) *Conclusion*. There is considerable irregularity in the curves but a distinct tendency to bimodal arrangement. There is some question as to interpretation. It is evident that the

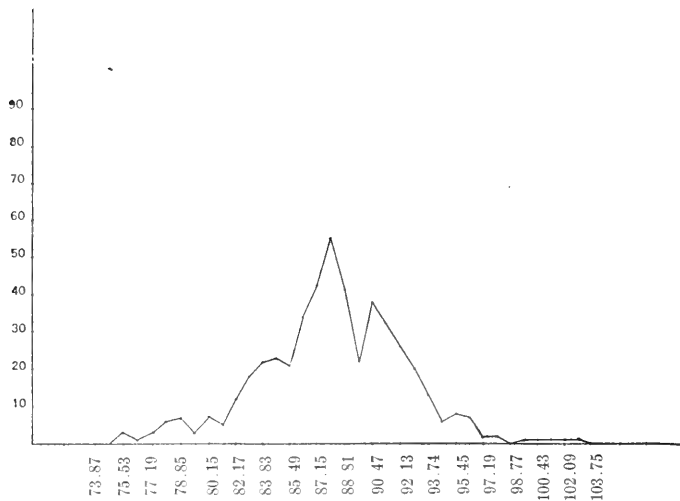


Fig. 32 *Melanoplus differentialis*; frequency distribution of head-lengths of 481 spermatozoa from left testis.

Value in μ	75.5	76.4	77.2	78.0	78.9	79.7	80.5	81.3	82.2	83.0
Frequency	3	1	3	6	7	3	7	5	12	18
	83.8	84.7	85.5	86.3	87.2	88.0	88.8	89.6	90.5	91.3
	22	23	21	34	42	55	41	22	38	32
	92.1	93.0	93.8	94.6	95.5	96.3	97.1	97.9	98.8	99.6
	26	20	13	6	8	7	2	2	0	1
	100.4									
	1									

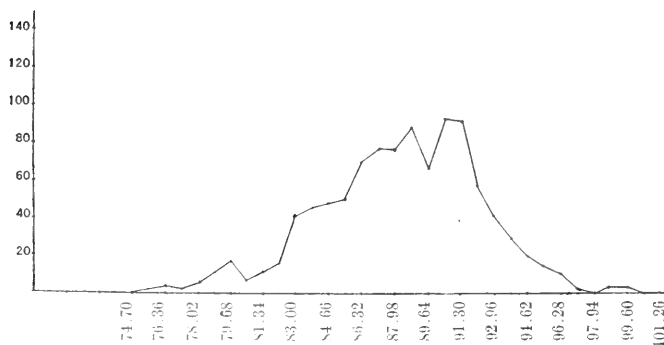


Fig. 33 *Melanoplus differentialis*; frequency distribution of head-lengths of 1008 spermatozoa from same left testis as the ones in figures 32 and 34.

Value in μ	73.9	74.7	75.5	76.4	77.2	78.0	78.9	79.7	80.5	81.3
Frequency	1	3	1	3	2	6	11	17	7	11
	82.2	83.0	83.8	84.7	85.5	86.3	87.2	88.0	88.8	89.6
	16	41	46	48	50	70	77	77	88	67
	90.5	91.3	92.1	93.0	93.8	94.6	95.5	96.3	97.1	97.9
	93	92	58	41	29	20	15	10	2	0
	98.8	99.6								
	3	3								

distribution is not that of a simple variation curve and undoubtedly several factors are involved in size determination. The three curves however show distinctly a tendency to a major bimodal subdivision. The minor modes may be due to inequalities in pairs of chromosomes that have no relation to the X chromosome or of course they may be due to any one or more of the sources of error mentioned on pages 196 to 201.

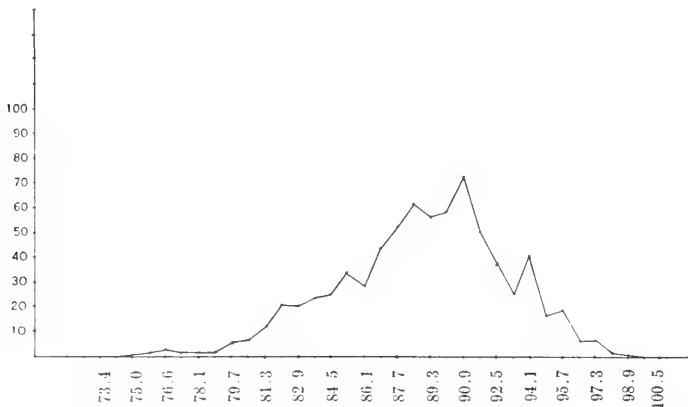


Fig. 34 *Melanoplus differentialis*; frequency distribution of head-lengths of 734 spermatozoa from same left testis as the ones in figures 32 and 33.

Value in μ	75.0	75.8	76.6	77.3	78.1	78.9	79.7	80.5	81.3	82.1
Frequency	1	2	3	2	2	2	6	7	12	21
	82.9	83.7	84.5	85.3	86.1	86.9	87.7	88.5	89.3	90.1
	21	24	25	34	29	44	53	62	57	59
	90.9	91.7	92.5	93.3	94.1	94.9	95.7	96.5	97.3	98.1
	73	51	38	26	41	17	19	7	7	2
	98.9									
	1									

9. *Gryllus abbreviatus*

(a) *Spermatogenesis evidence concerning dimorphism.* The chromosomal relations for this species are not known. Another species of the same genus, *G. domesticus*, has an X chromosome, according to W. J. Baumgartner ('05).

(b) *Material and method.* Obtained at Douglas Lake, Michigan, August 23, 1913; motile in Ringer's solution; killed in osmic fumes; diagram of sperm-head in figure 10 c; measure-

ments were to halves of ocular divisions, one division being equal to 0.7μ .

(c) *Data.* Figure 35; 552 spermatozoa from the right testis.

(d) *Conclusion.* The curve is unimodal. It is not possible to tell by inspection whether it is a simple normal variation curve (fig. 1) or a combination of two such curves with modes close together (fig. 6).

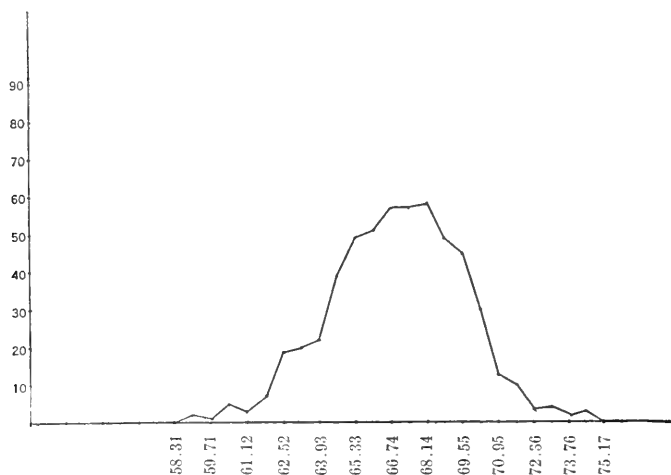


Fig. 35 *Gryllus abbreviatus*; frequency distribution of head-lengths of 552 spermatozoa from the right testis.

Value in μ	59.0	59.7	60.4	61.2	61.8	62.5	63.2	63.9	64.6	65.3
Frequency	2	1	5	5	7	19	20	22	39	49
	66.0	66.7	67.4	68.1	68.9	69.6	70.3	71.0	71.7	72.4
	51	57	57	58	49	45	30	13	10	4
	73.1	73.8	74.5							
	4	2	3							

10. *Aeshna canadensis*

(a) *Spermatogenesis evidence concerning dimorphism.* The chromosomal relations are not known for this species, but in another dragon fly, *Anax junius*, Lefevre and McGill ('08) have described an X chromosome. The following measurements are taken from their figure 4 e.

Chromosomes	Width	Length	Chromosomes	Width	Length
a	18	23	h	27	33
b	20	30	i	21	28
c	21	27	j	20	27
d	14	16	k	19	28
e	20	27	l	20	26
f	18	25	m	24	34
g	16	21	X	23	31

These give expected ratios of 1.00 : 1.07 on the basis of complete fusion of chromosomes and production of spermatozoa of like shape and 1.00 : 1.09 for end to end fusion of chromosomes.

(b) *Material and method.* Obtained at Douglas Lake, Michigan, August 23, 1913; killed in osmic fumes; diagram of sperm-head in figure 10 d; measurements are to quarters of an ocular division, one division being equal to 1.4μ .

(c) *Data.* Figure 36; 496 spermatozoa from the right testis. The distribution is bimodal but unequal. The two modes are at 50.2μ and 51.6μ , giving a ratio of 1.00 : 1.03. This is considerably less than the ratio 1.00 : 1.07 or 1.00 : 1.09, that would be expected for *Anax junius*.

(d) *Conclusion.* The presence of two size groups is indicated by the measurements.

11. *Rana pipiens*

(a) *Spermatogenesis evidence concerning dimorphism.* While a number of investigators have studied the spermatogenesis of the frog no chromosomal differences have so far been made out between two groups of spermatids.

(b) *Material and method.* Material obtained August 24, 1913, at Douglas Lake, Michigan; motile; killed in osmic fumes; diagram of sperm-head in figure 10 l; measurements are to tenths of a micrometer division, one division being equal to 0.96μ .

(c) *Data.* Figure 37 gives the frequency distribution of 494 spermatozoa from a left testis. Each group covers three-tenths of a micrometer division. The distribution is bimodal with the smaller individuals less abundant than the larger. The modes are at 9.74μ and 10.60μ , giving a ratio of 1.00 : 1.09.

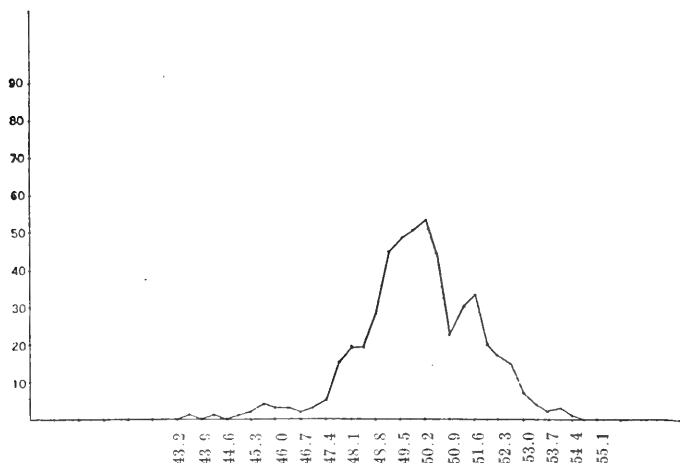


Fig. 36 *Aeshna canadensis*; frequency distribution of head-lengths of 496 spermatozoa from the right testis.

Value in μ	42.2	42.5	42.9	43.2	43.6	43.9	44.3	44.6	45.0	45.3
Frequency	1	0	0	0	1	0	1	0	1	2
	45.7	46.0	46.4	46.7	47.1	47.4	47.8	48.1	48.5	48.8
	4	3	3	2	3	5	15	14	19	28
	49.2	49.5	49.9	50.2	50.6	50.9	51.3	51.6	52.0	52.3
	44	48	50	53	44	23	30	33	20	17
	52.7	53.0	53.4	53.7	54.1	54.4				
	15	7	4	2	3	1				

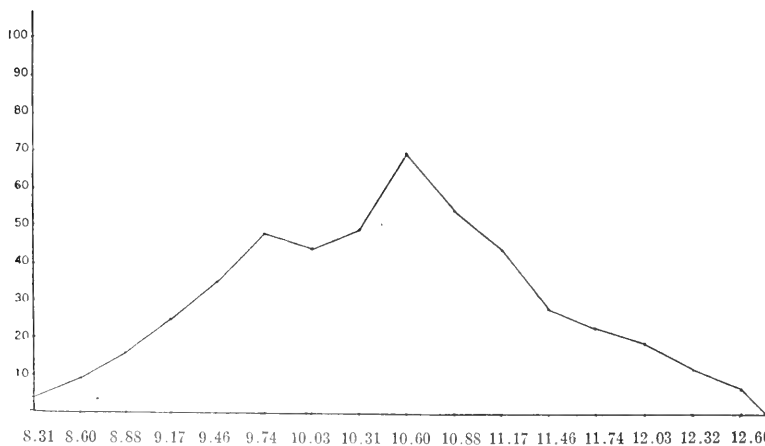


Fig. 37 *Rana pipiens*; frequency distribution of head-lengths of 494 spermatozoa from a single testis.

Value in μ	7.74	8.03	8.31	8.60	8.88	9.17	9.46	9.74	10.03	10.31
Frequency	3	2	4	9	16	25	35	48	44	49
	10.60	10.88	11.17	11.46	11.74	12.03	12.32	12.60	12.89	
	69	54	44	28	23	19	12	7	3	

(d) *Conclusion.* The existence of the bimodal condition in this species can not be ascribed to any known chromosomal differences.

12. *Pseudemys troosti*

(a) *Spermatogenesis evidence concerning dimorphism.* There is no description for this species but according to H. E. Jordan ('14) several species of turtles have an accessory chromosome.

(b) *Material and method.* Material obtained at Urbana, Illinois, November 8, 1913; motile in Ringer's solution killed in Bouin's fluid; diagram of sperm-head in figure 10 k; measurements to quarters of a micrometer division in set A and to tenths in set B. In set A one micrometer division equals 0.95μ and in set B 0.955μ . The spermatozoa are slightly curved in life, but when smeared on a slide many become perfectly straight. The preparations that were most satisfactory as regards straightness of the spermatozoa were those taken several hours after the testis was removed from the animal and when most of the spermatozoa had stopped moving.

(c) *Data.* Set A, figure 38, gives the data for 501 spermatozoa from a single testis. These give a symmetrical bimodal distribution with smooth slopes. There are, however, a few spermatozoa which are much larger than the average. The two modes are at 10.43μ and 10.91μ , giving a ratio of 1.00 : 1.05. Set B, figure 39, gives the data for 487 spermatozoa from the same testis as figure 38. There is a marked division into two principal groups, but each of these has a secondary sub-division. The principal modes are at 10.50μ and 11.36μ , giving a ratio of 1.00 : 1.08.

(d) *Conclusion.* There can be no doubt of the presence of two principal size groups. Jordan described an accessory chromosome for some of the turtles but as no figures are published it is not possible to tell whether or not the modes agree with the comparative sizes to be expected on the basis of chromosomal differences.

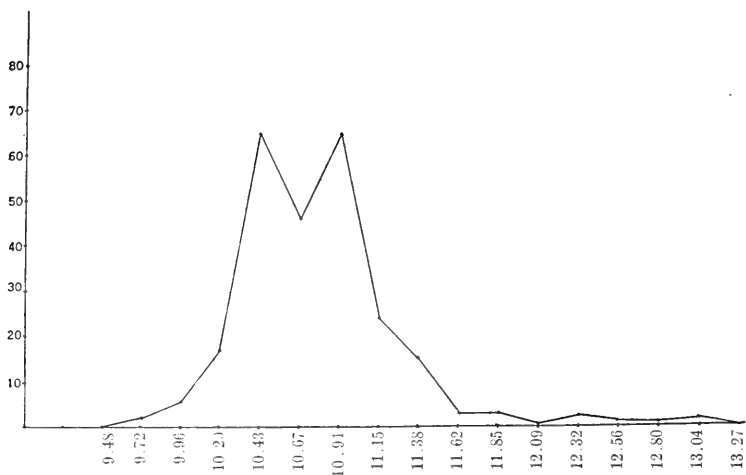


Fig. 38 *Pseudemys troosti*; frequency distribution of head-lengths of 501 spermatozoa from a single testis.

Value in μ	9.72	9.96	10.20	10.43	10.67	10.91	11.15	11.38	11.62
Frequency	4	11	33	129	92	129	48	30	6
	11.85	12.09	12.32	12.56	12.80	13.04	13.27		
	6	1	5	2	2	3	0		

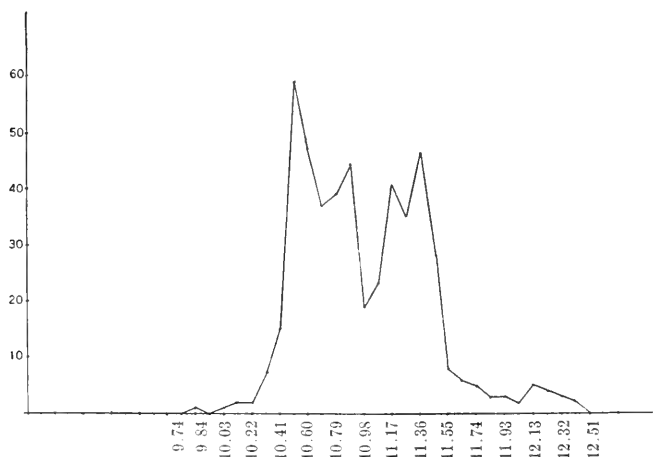


Fig. 39 *Pseudemys troosti*; frequency distribution of head-lengths of 487 spermatozoa from the same testis as the one in figure 38.

Value in μ	9.84	9.93	10.03	10.12	10.22	10.31	10.41	10.50	10.60
Frequency	1	0	1	2	2	7	15	59	47
	10.69	10.79	10.88	10.98	11.08	11.17	11.27	11.36	11.46
	37	39	44	19	23	41	35	46	28
	11.55	11.65	11.74	11.84	11.93	12.03	12.13	12.22	12.32
	8	6	5	3	3	2	5	4	3
	12.41								
	2								

13. Ovis aries

(a) *Spermatogenesis evidence concerning dimorphism.* According to H. E. Jordan ('13) an X chromosome is present.

(b) *Material and method.* Material obtained at Chicago, Illinois, July, 1913; motile; killed in Gilson's mercurio-nitric fluid; diagram of sperm-head in figure 10 o; measurements are to tenths of ocular division, one division being equal to 0.96μ .

(c) *Data.* Figure 40 gives the distribution for 498 spermatozoa plotted by classes of two-tenths of an ocular division. The distribution is bimodal with modes at 5.94μ and 6.37μ , giving a ratio of 1.00 : 1.07.

(d) *Conclusion.* Two size groups are indicated by the measurements.

14. Bos taurus

(a) *Spermatogenesis evidence concerning dimorphism.* There is an accessory chromosome, according to H. E. Jordan ('13).

(b) *Material and method.* Obtained at Chicago, Illinois, July, 1913; motile; killed in Gilson's fluid; diagram of sperm-head in figure 10 n; measurements were made to tenths of an ocular division, one division being equal to 0.96μ in set A and 0.97μ in set B.

(c) *Data.* Set A; figure 41 gives the distribution for 606 sperm-head lengths. The curve is bimodal with modes at 8.05μ and 8.33μ , giving a ratio of 1.00 : 1.035. Set B, figure 42, gives the frequency distribution of areas of the flat surface of the sperm-heads. 502 individuals were measured for length and width. The areal ratios were obtained by multiplying for each individual case the length by the width. Plotting these pseudo-areas the distributions shown in the figure are obtained. The result indicates that the population consists of more than one group. Four modal points are present, but the explanation of this condition is not at hand.

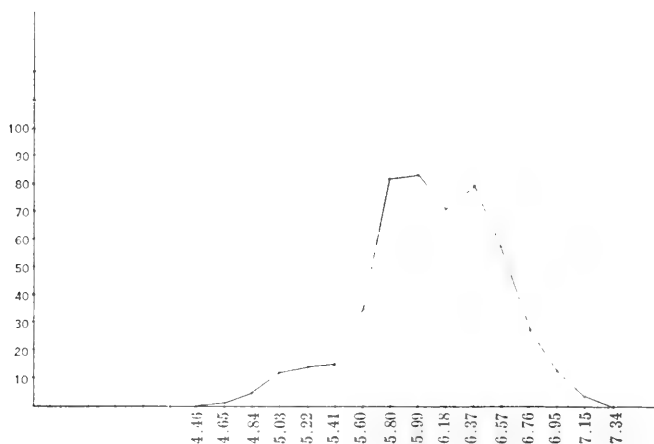


Fig. 40 *Ovis aries*; frequency distribution of head-lengths of 498 spermatozoa from a single testis.

Value in μ	4.64	4.83	5.02	5.21	5.40	5.60	5.79	5.98	6.17	6.36
Frequency	1	5	12	14	15	34	82	83	71	79
	57	28	13	4						

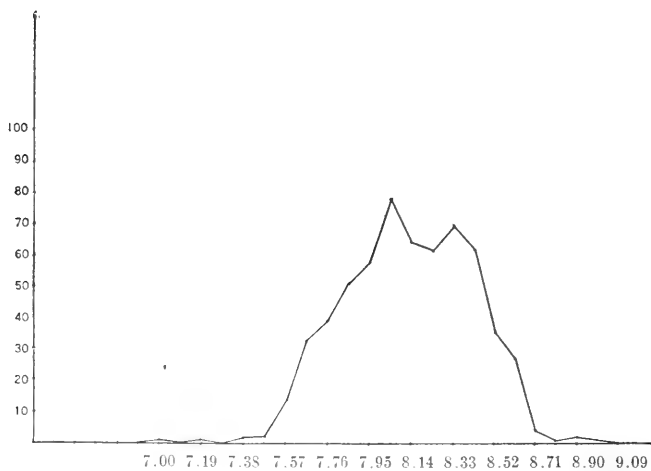


Fig. 41 *Bos taurus*; frequency distribution of head-lengths of 606 spermatozoa from a single testis.

Value in μ	7.00	7.10	7.19	7.28	7.38	7.47	7.57	7.66	7.76	7.86
Frequency	1	0	1	0	2	2	14	33	39	51
	7.95	8.05	8.14	8.24	8.33	8.43	8.53	8.62	8.71	8.81
	58	78	64	62	69	62	35	27	4	1
	8.90	9.00								
	2	1								

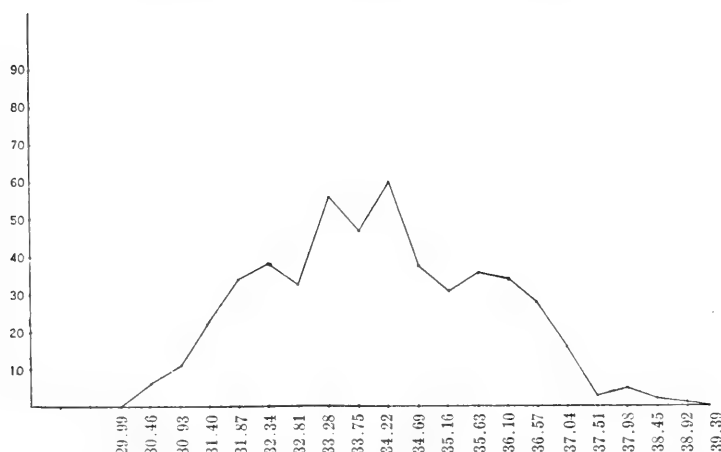


Fig. 42 *Bos taurus*; frequency distribution of head-areas of 502 spermatozoa from the same testis as the one in figure 41.

Value in μ	30.5	30.9	31.4	31.9	32.3	32.8	33.3	33.8	34.2	34.7
Frequency	6	11	23	34	38	53	56	47	60	38
	35.2	35.6	36.1	36.6	37.0	37.5	38.0	38.5	38.9	
	51	36	34	28	16	3	5	2	1	

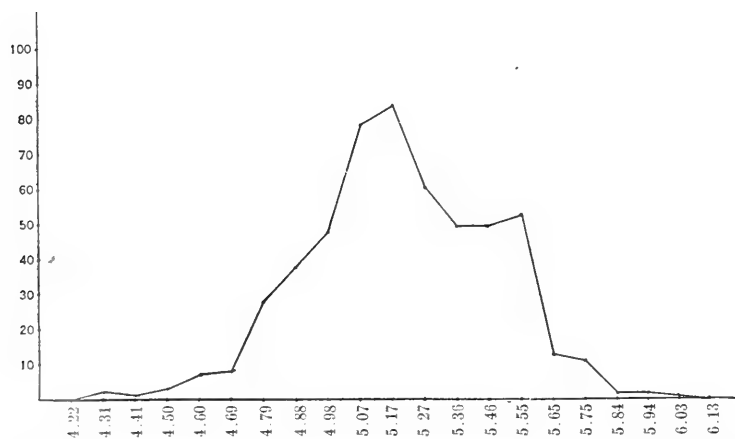


Fig. 43 *Canis familiaris*; frequency distribution of head-lengths of 540 spermatozoa from a single testis.

Value in μ	4.31	4.41	4.50	4.60	4.69	4.79	4.88	4.98	5.07	5.17
Frequency	2	1	3	7	8	28	33	48	78	84
	5.27	5.36	5.46	5.55	5.65	5.75	5.84	5.94	6.03	
	61	50	50	53	13	11	2	2	1	

15. Canis familiaris

(a) *Spermatogenesis evidence concerning dimorphism.* According to H. E. Jordan ('13) an accessory chromosome is present.

(b) *Material and method.* Material obtained in Chicago, Illinois, July, 1913; motile; killed in Gilson's fluid; diagram of sperm-head shown in figure 10 p; measurement to tenths of an ocular division, one division being equal to $.97\mu$.

(c and d) *Data and conclusion.* Figure 43 gives the frequency distribution for 540 spermatozoa. The curve shows two modes and indicates the presence of two size groups, one larger than the other. The modes are at 5.17μ and 5.55μ yielding a ratio of 1.00 : 1.07.

DISCUSSION

The hypothesis that two kinds of spermatozoa are produced, one determining maleness and the other femaleness, needs experimental confirmation. Such a confirmation is not to be expected as long as the recognition of the two kinds is confined to chromosomal differences during the spermatocyte divisions. It is necessary to devise some means of distinguishing the spermatozoa of the two groups from each other after they have become functional. Since in most of the cases spermatogenesis studies have shown that the two groups differ in amount of chromatin material received, and since the sperm-head is made up almost wholly of chromatin a size difference between the mature functional spermatozoa is to be expected. If such a difference is proven to exist it should be possible to separate the larger spermatozoa from the smaller ones while they are still living, and determine whether or not there is a difference between them as regards sex production.

The present paper gives data which show that there is a wide range in length of the sperm-head in completed spermatozoa and that the distribution among the different sizes is such as to demonstrate the presence of two size groups.

From the published drawings of the chromosomal histories in several of the species a rough calculation was made of the

TABLE 1

Showing expected and observed ratios between lengths of sperm-heads. The expected ratios are those calculated on the basis of the most probable condition, the production of spermatozoa which have the same shape regardless of size

NAME	FIGURE	NUMBER OF SPERMATOOA	LOWER MODE IN MICRONS	UPPER MODE IN MICRONS	OBSERVED RATIO	EXPECTED RATIO
Musca domestica.....	11	444	35.80	38.60	1.00 : 1.080	1.00 : 1.080
Musca domestica.....	12	465	35.80	38.60	1.00 : 1.080	1.00 : 1.080
Musca domestica.....	13	769	36.40	38.00	1.00 : 1.040	1.00 : 1.080
Lygaeus turcicus.....						1.00 : 1.020
Lygaeus kalmii.....	14	493	36.80	38.30	1.00 : 1.040	
Lygaeus kalmii.....	15	501	36.80	37.80	1.00 : 1.030	
Alydus pilosulus.....	16	429	31.90	33.90	1.00 : 1.060	1.00 : 1.060
Alydus pilosulus.....	17	469	32.25	33.75	1.00 : 1.050	1.00 : 1.060
Anasa tristis.....	18	653	28.35	30.20	1.00 : 1.070	1.00 : 1.110
Anasa tristis.....	19	391	27.50	30.20	1.00 : 1.100	1.00 : 1.110
Anasa tristis.....	20	370	28.10	30.80	1.00 : 1.100	1.00 : 1.110
Anasa tristis.....	21	443	27.50	30.80	1.00 : 1.120	1.00 : 1.110
Anasa tristis.....	22	404	28.10	30.80	1.00 : 1.100	1.00 : 1.110
Anasa tristis.....	23	384	28.60	30.80	1.00 : 1.080	1.00 : 1.110
Anasa tristis.....	24	394	28.10	30.80	1.00 : 1.100	1.00 : 1.110
Anasa tristis.....	25	322	30.80	31.80	1.00 : 1.030	1.00 : 1.110
Anasa tristis.....	26	993		31.50		1.00 : 1.110
Anasa tristis.....	27	444	28.40	31.50	1.00 : 1.110	1.00 : 1.110
Trirhabda virgata.....						1.00 : 1.060
Trirhabda tomentosa.....	28	481	17.02	17.78	1.00 : 1.045	
Phytonomus punctatus.....	29	506	33.00	34.00	1.00 : 1.030	
Phytonomus punctatus.....	30	507	33.30	35.30	1.00 : 1.060	
Melanoplus femur-rubrum....	31	491	80.50	83.00	1.00 : 1.030	
Melanoplus differentialis....	32	481	88.00	90.50	1.00 : 1.030	
Melanoplus differentialis....	33	1008	88.80	90.50	1.00 : 1.020	
Melanoplus differentialis....	34	734	88.50	90.90	1.00 : 1.030	
Gryllus abbreviatus.....	35	552				
Anax junius.....						1.00 : 1.070
Aeshna canadensis.....	36	496	50.20	51.60	1.00 : 1.030	
Rana pipiens.....	37	494	9.74	10.60	1.00 : 1.090	
Pseudemys troosti.....	38	501	10.43	10.91	1.00 : 1.050	
Pseudemys troosti.....	39	487	10.50	11.36	1.00 : 1.080	
Ovis aries.....	40	498	5.94	6.37	1.00 : 1.070	
Bos taurus.....	41	606	8.05	8.33	1.00 : 1.035	
Bos taurus (area).....	42	502				
Canis familiaris.....	43	540	5.17	5.55	1.00 : 1.070	

expected ratio between the lengths of the sperm-heads in the two groups on the supposition that amount of chromatin material received is an important factor in the determination of the size of the resultant sperm-heads. Likewise from the measurement of the actual lengths of the sperm-heads the ratio between the values of the modes of the two size groups was obtained. The ratios obtained in these two ways agree to such an extent as to make it almost beyond question that there is a causal connection between the two size groups and the two groups based on chromosomal differences. It thus becomes possible to apply experimental test to the hypothesis that these chromosomal differences are of sex-determining value. The larger spermatozoa ought to yield a preponderance of females, and the smaller ones a preponderance of males in case the hypothesis is correct.

Certain of the facts concerning the two size groups are of importance in any experimental application of the results. The two groups are not wholly distinct. Spermatozoa of medium size may belong to either of the two size groups, making up the general population. As one goes to the smaller and smaller spermatozoa in the one direction, and to the larger and larger ones in the other, the certainty increases that apparent position of any individual spermatozoon is a good criterion of the actual group to which it belongs. For the largest as for the smallest spermatozoa the certainty ought to be almost complete. Any separation method carried on with living material should therefore, as far as possible, concern itself primarily with spermatozoa near the extremes. Fortunately the extreme individuals should be the most easily separable.

While the validity of the general conclusion seems clear the data are not sufficiently accurate to make possible a definite explanation of the peculiarities of size distribution in individual sets of measurements. After the full discussion of the possible sources of error, on pages 196 to 201, it would be out of place to consider them further here. No doubt it is unwise to attempt a discussion of such observed facts as the inequality of the two groups in many of the cases, and the reciprocal relation between right and left testes of two individuals of *Anasa tristis*. Like-

wise there is no valid ground at present for a consideration of the numerous other factors besides chromatin content that must effect size of spermatozoa. What these factors are is conjectural, but they certainly act within both of the main size groups, and by no means invalidate the conclusion of the existence of the factor that determines the main difference between the two groups. Also it is unnecessary to comment here on the numerous observations that have been made on kinds of dimorphism in spermatozoa of a character quite different from the present. These bear only indirectly upon the problem in hand. In most of the species described in this paper, as the data show, a very small proportion of the whole number may be classed as giant spermatozoa, except for the fact that there are usually all gradations in size between them and the smaller individuals.

SUMMARY

1. Spermatogenesis studies have shown for a great many species of animals that two kinds of spermatids are produced differing from each other in chromatin content, and that this difference is probably of sex-determining value. In order that experimental test of this hypothesis may be made it is necessary to distinguish the two kinds when they have become functional spermatozoa.

2. Since, as spermatids, the groups receive different amounts of chromatin, and since the sperm-heads are almost wholly made up of chromatin it follows that the spermatozoa ought to belong to two principal size groups.

3. The present paper is a study of size differences in length of the head in completed spermatozoa from single testes in fifteen species of animals, with the object of determining the existence or non-existence of two size groups correlated with the chromosomal difference.

4. A considerable variation in length of the spermatozoa from a single testis was discovered in each case, and the existence of two size groups could be determined only by measuring large numbers of spermatozoa and plotting the size distribution.

Thirty-three such separate determinations were made with an average of about 523 measurements for each determination, and a total of 17,252 for the whole.

5. In nearly all cases there were two high points or modes in the distribution curve indicating that the population of spermatozoa is made up of two separate groups.

6. A comparison of the degree of separation of these modes with the expected degree as derived from a calculation based on the chromosomal histories shows in general a striking correspondence.

7. The general conclusion is drawn that two size groups may be distinguished in many of the species which show chromosomal differences in spermatogenesis, and that the size difference is based on the difference in chromosomal content.

8. If the hypothesis that the chromosomal differences are of sex-determining value is true it follows that the larger spermatozoa differ from the smaller ones in sex determination.

9. While the method seems competent to yield a conclusive general result no attempt is made to minimize the many possible sources of error liable to come into individual determinations. The results for the individual species follow. In each case where known, there is given the expected ratio between the lengths of the two groups of spermatozoa on the basis of chromosomal material received, assuming that the completed spermheads are of the same shape regardless of size. Then follow the observed ratios between the two modes of the distribution curve. Two size groups are of course present unless otherwise stated.

10. *Musca domestica*: The expected ratio is 1.00 : 1.08, and the observed ratios are 1.00 : 1.08, 1.00 : 1.08 and 1.00 : 1.04.

11. *Lygaeus kalmii*: No chromosomal data for this species are available. The expected ratio for *Lygaeus turcicus* is 1.00 : 1.02 and the observed ratios for *L. kalmii* are 1.00 : 1.03 and 1.00 : 1.04.

12. *Alydus pilosulus*: The expected ratio is 1.00 : 1.06 and the observed ratios are 1.00 : 1.06 and 1.00 : 1.05.

13. *Anasa tristis*: Two size groups are indicated in nine of the ten determinations. The expected ratio is 1.00 : 1.11 and the

observed ratios are 1.00 : 1.07, 1.00 : 1.10, 1.00 : 1.10, 1.00 : 1.12, 1.00 : 1.10, 1.00 : 1.08, 1.00 : 1.10, 1.00 : 1.03 and 1.00 : 1.11.

14. *Trirhabda tomentosa*: No chromosomal data are available for this species. The expected ratio for *Trirhabda virgata* is 1.00 : 1.06 and the observed ratio for *T. tomentosa* 1.00 : 1.045.

15. *Phytonomus punctatus*: The observed ratios are 1.00 : 1.03 and 1.00 : 1.06.

16. *Melanoplus femur-rubrum*. There are subsidiary modes besides the two principal ones. The observed ratio between the principal modes is 1.00 : 1.03.

17. *Melanoplus differentialis*. There are subsidiary modes besides the two principal ones. The observed ratios between the principal modes are 1.00 : 1.03, 1.00 : 1.02 and 1.00 : 1.03.

18. *Gryllus abbreviatus*. The curve of distribution is uni-modal, and indicates the presence either of one size group or of two groups so close together as to simulate a single group when combined. The chromosomal history shows two groups of spermatids, but the figures do not allow of calculations.

19. *Aeshna canadensis*. No chromosomal data for this species are available. The expected ratio for another dragon-fly, *Anax junius*, is 1.00 : 1.07. The observed ratio for *Aeshna canadensis* is 1.00 : 1.03.

20. *Rana pipiens*. No chromosomal differences have been made out, but the spermatozoa show two size groups with a ratio of 1.00 : 1.09.

21. *Pseudemys troosti*. The observed ratios are 1.00 : 1.05 and 1.00 : 1.08.

22. *Ovis aries*. The observed ratio is 1.00 : 1.07.

23. *Bos taurus*. The observed ratio is 1.00 : 1.035.

24. *Canis familiaris*. The observed ratio is 1.00 : 1.07.

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THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF A MENDELIAN CHARACTER

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FORTY-TWO FIGURES (FIVE PLATES)

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INTRODUCTION

A new mutation in *Drosophila ampelophila*, which took the form of reduplication in the legs, appeared in a selection experiment. It was inherited, although at first it did not seem to give the ordinary Mendelian ratios. Many unsuccessful efforts to obtain pure stock were made, and, even after selection for many generations, a single pair of abnormal flies would produce both normal and abnormal offspring. It finally became clear that a low temperature was necessary for the realization of the abnormal type. The stock which had undergone mutation was the only one to develop the reduplications when subjected to a low temperature. A definite sex-linked factor was found to be responsible for the inheritance of this peculiarity.

I wish to thank Dr. T. H. Morgan, under whose direction the work was carried on, for his many helpful suggestions and valuable criticisms.

EFFECT OF SELECTION

DeVries and Johannsen hold that, in the absence of mutation, selection can do nothing but sort out from a population characteristics already existing. According to this conception, any population may be made up of a series of forms, and this series may be broken up into smaller groups by selection, but the limit of variability can not be transcended. The most extreme individuals in a race produced by such a sorting-out process are no more extreme than the most extreme in the heterogeneous population. Recently Castle and others have endeavored to show that selection may affect the degree of development of the character selected.

My work began with a study of the effect of selection on the number of 'teeth' in the sex-comb of *Drosophila*. The sex-comb is confined to the male, and is situated on the first tarsal segment of the first pair of legs. The 'teeth' composing the sex-comb are modified bristles. As the female has no sex-comb, the selection was necessarily confined to the males, and it was further restricted to the comb on the right leg. The legs of fifty wild males were examined, and the teeth in the combs counted. The average number was found to be 10. At the close of the selection another count of wild flies was made and the number was found to be nearly the same, viz., 9.9.

TABLE 1

Showing average number of teeth in sex-comb; obtained by selection for high number of teeth for seven generations

P	F ₁	F ₂	F ₃	F ₄
11.00	10.91	11.16	11.35	11.78
F ₅		F ₆		F ₇
11.86		11.41		11.56
11.88		11.50		11.30
11.67 av. 11.66		12.15 av. 11.85		11.50
11.58		12.70		11.42
11.33		11.50		11.38 av. 11.37
5 bottles		5 bottles		11.42
				11.22
				11.34
				11.18
				9 bottles

Twenty-four individuals from the wild stock were mated in pairs to wild females. Most of the males selected had eleven teeth. As one of the twenty-four lines yielded better results than any of the others, selection was continued in this line only. The average number of teeth in F₁ was 10.91. Those with the highest number of teeth in the comb were selected for breeding. They were mated to sister females. The average number of teeth of their sons was 11.16. Those lines which had failed in F₂ to be high producers were thrown away. Selection was

continued for seven generations, sisters of the males being used in each mating. After the F_1 , the average number of teeth showed a steady increase, the highest number—11.85—being reached in the sixth generation. Some bottles in this generation produced an average of over 12 teeth. The mean number in the seventh generation fell somewhat lower—11.37. However, the average number of teeth in this generation, the last one bred,

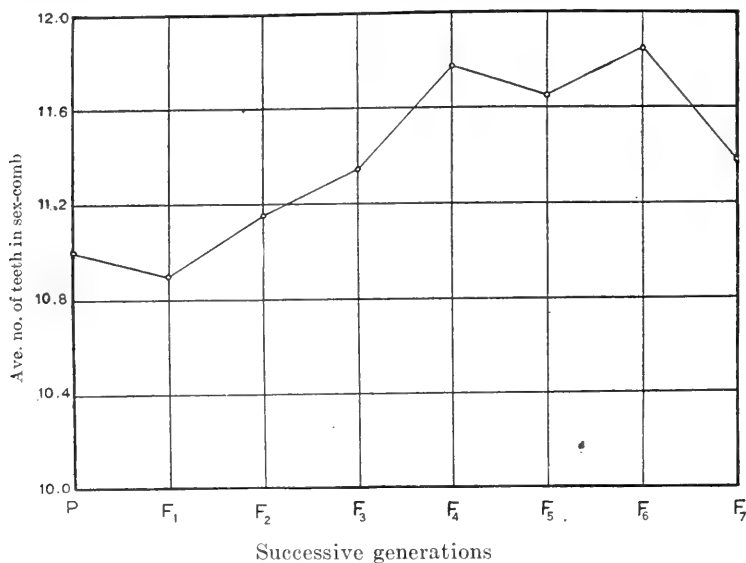


Diagram 1

was nearly 0.4 higher than that of the ancestor (11) and was 1.37 higher than that of the average wild fly. The results of the selection are plotted in diagram 1.

Artificial selection has thus brought about a line of flies having a larger sex-comb than that of the average wild fly. The result is in harmony with the view that selection is only a sorting out process, for although the average had been raised, yet the largest number of teeth in the selected line was no higher than the largest number in the wild stock.

A MUTATION CAUSING REDUPLICATION IN THE LEGS

1. *The origin of the mutation*

When the selection had proceeded to the fifth generation, several abnormal males appeared in the stock. The abnormalities were all connected with reduplication of leg or tarsal segments. In one of the mutants, the left leg was divided, nearly to its base, into two parts, each with a perfect sex-comb. On the right tarsus of the same fly were two combs, one placed above the other. The other mutants showed various kinds of reduplication in the appendages. They were bred to sisters; of the F_1 , 13.9 per cent were abnormal. The F_1 normals were inbred and abnormalities in the legs appeared again in F_2 . The F_2 normals were inbred; and later the F_3 normals and F_3 abnormals. The result was as follows:

From P.....	13.9 per cent abnormals in F_1
F_1 normal.....	0.3 per cent abnormals in F_2
F_2 normal.....	1.7 per cent abnormals in F_3
F_3 normal.....	1.7 per cent abnormals in F_4
F_3 abnormal.....	2.02 per cent abnormals in F_4

The F_3 normals produced nearly as many abnormals as did the F_3 abnormals. Among the abnormal flies there was a wide range of variation, but usually only one or two extra parts were found in a single fly. The abnormalities occurred most frequently in either the left or the right anterior leg, but the other legs also were sometimes affected. At times the tarsus alone was affected, and at other times the leg as well. Often only one leg was affected but sometimes both anterior legs and sometimes nearly all the legs showed reduplications. The first mutants were males, and only after much inbreeding were abnormal females obtained.

In order to test whether there might be in the cultures some special condition that caused the abnormalities, a stock bottle was allowed to become dry, and wild flies were then transferred to it with fresh food. When their progeny hatched, no abnormal flies were found among them.

2. Specific types of abnormalities not inherited

Since the number of abnormal flies obtained by inbreeding was small, and since there was such a variety in the form assumed, it seemed at first possible that many factors might be involved. Attempts were therefore made to obtain different lines, each with a specific kind of reduplication. Thus, two flies having a bifid first right leg were mated together, or similarly, two flies having a left duplex tarsus. In the following cases of this kind, only the abnormal offspring will be considered:

(1) Two flies, both with right first leg affected, produced among the F_1 4 abnormals, all with left first leg affected.

(2) Six flies with either right first, left first, or both right first and left first affected were mated. They produced 11 abnormals, as follows:

- 4 flies with reduplication in right first
- 4 flies with reduplication in left first
- 1 fly with reduplication in right and left first
- 1 fly with reduplication in left second
- 1 fly with reduplication in right first and right second

(3) Three F_1 flies (2♀, 1♂) all with right first affected produced:

- 3 flies with reduplication in right first
- 3 flies with reduplication in left first
- 1 fly with reduplication in right third
- 1 fly with reduplication in right second, left first

(4) Parents—males and females with right first or left first, or both, affected produced 7 abnormals as follows:

- 2 flies with reduplication in left first
- 2 flies with reduplication in right first
- 1 fly with reduplication in right second
- 1 fly with reduplication in right first and right third
- 1 fly with reduplication in right first and left first

(5) Parents abnormal—with reduplication in right first or left first produced 42 abnormals as follows:

- 5 flies with reduplication in right first
- 7 flies with reduplication in left first
- 4 flies with reduplication in left third
- 2 flies with reduplication in right third
- 2 flies with reduplication in left second
- 1 fly with reduplication in left third, right second
- 1 fly with reduplication in right first, right third

(6) Parents—males and females with right first affected produced 9 abnormals, viz:

- 4 flies with reduplication in left first
- 2 flies with reduplication in left third
- 1 fly with reduplication in left first, right first
- 1 fly with reduplication in right first
- 1 fly with reduplication in left second

The results thus indicate that the particular abnormalities which appear in the offspring of abnormal flies have no relation to the particular abnormalities of the parents.

3. The apparently changing dominance of the new character

When bred to wild females, the abnormal males produced all normals in F_1 . In F_2 there were 1110 normals and 17 abnormals. The abnormality acted apparently as a recessive character. Its appearance in the F_1 when the original male mutants were bred to sisters was thought to be due to the heterozygous condition of the females.

To obtain pure stock I selected the abnormal flies throughout the summer of 1912. In each generation, the abnormals were separated and mated to other abnormals. The work was done both in pairs and in mass cultures. The per cent of abnormal individuals varied greatly from one generation to another, but never approximated to 100 per cent.

In the fall of 1912, after many apparently unsuccessful attempts to get pure stock, I began experimental work. Crosses with the wild gave the results in table 2. Abnormal males were mated with wild females and abnormal females with wild males. Since a few abnormal males and females appeared in the F_1 of both

crosses, it seemed that the factor causing reduplication was not sex-linked, and not always recessive to the wild, but on the other hand, if the character was a non-sex-linked dominant, it was difficult to see why such a small number of abnormals appeared.

Back-crosses were then made by mating the F_1 flies recorded in table 2 both with abnormal and with wild flies. These crosses

TABLE 2

CROSS I

<i>P</i> Abnormal ♂ by normal (wild) ♀							Total	
F ₁	{	Normal ♂.....	27	85	84	122	114	432
		Normal ♀.....	15	99	100	151	140	505
		Abnormal ♂.....			3		6	9
		Abnormal ♀.....		1				1
F ₂	{	Normal ♂.....	229	126		16	101	472
		Normal ♀.....	265	136		38	173	612
		Abnormal ♂.....	13	7		3	18	41

CROSS II

<i>P</i> Abnormal ♀ by normal (wild) ♂						Total
F_1	Normal ♂.....		33	34	93	160
	Normal ♀.....		45	84	151	280
	Abnormal ♂.....			19	33	52
	Abnormal ♀.....			1	12	13
F_2	Normal ♂.....			72		
	Normal ♀.....			114		
	Abnormal ♂.....			26		
	Abnormal ♀.....			2		

CROSS III

<i>P</i> Abnormal red ♂ by normal white ♀						Total
F_1	Normal white ♂.....	10	96	89		195
	Normal red ♀.....	15	107	73		195
	Normal red ♂.....	27	57	40		124
	Normal white ♂.....	23	69	46		138
F_2	Normal red ♀.....	35	95	80		210
	Normal white ♀.....	35	85	78		198
	Abnormal red ♂.....	1	17	3		21
	Abnormal white ♂.....	1	15	4		10

again made it clear that abnormality behaves differently at different times. The following cases illustrate this. From two of the back-crosses (table 3, Series b) in which normal F_1 flies were mated to wild flies, F_2 abnormalities resulted. The F_1 flies had only one dose of the factor and were normal, yet some of their offspring had one dose and were abnormal. A normal

TABLE 3

Series a P Abnormal ♂ by wild ♀ (Crosses I and III of table 2)

	F_1 Normal ♂ by wild ♀ (5 bot- tles)	F_1 Normal ♂ by abnormal ♀ (5 bottles)	F_1 Normal ♀ by abnormal ♂ (3 bottles)
F_2 { Normal ♂.....	222	49*	157
Normal ♀.....	269	228	171
Abnormal ♂.....		67	30
Abnormal ♀.....			13

Series b P Abnormal ♀ by wild ♂ (Cross II of table 2)

	F_1 Normal ♂ by wild ♀ (3 bottles)	F_1 Normal ♀ by wild ♂ (2 bottles)	F_1 Normal ♀ by abnormal ♂ (1 bottle)
F_2 { Normal ♂.....	229	22	21
Normal ♀.....	230	121	34
Abnormal ♂.....	18†	28	29
Abnormal ♀.....		3	53

* One of these 49 normal males was bred to a wild female and produced abnormal flies in the next generation. This normal male was thus potentially abnormal.

† These 18 males were all from one of the three bottles.

F_2 male from Series a was tested for abnormality by breeding to a wild female, and abnormal flies were produced in the next generation. The F_2 male though normal, must have carried the factor for abnormality. An F_1 normal female (Series b) was back-crossed to an abnormal male. Over half the flies of F_2 were abnormal, though only half could have been homozygous. The F_1 female must have been heterozygous and yet she was normal. It is evident, then, that a fly may be heterozygous and either normal or abnormal, and in other words, the character may sometimes act as a dominant and sometimes as a recessive.

4. *Sex-linkage of the new factor, A, at first obscured by the phenomenon of non-disjunction*

Though the first work seemed to indicate that the factor for abnormality, which we will call A, was not sex-linked, the back-crosses threw doubt on this conclusion. For example, F_1 normal males, from cross abnormal male by wild female, were mated to wild females (table 3, Series a). From this cross were hatched 491 flies, and these were all normal. If the A factor is not sex-linked, why did the F_1 males not transmit it to any of their offspring? If it is sex-linked, then none of the F_1 males used in this cross carried the factor, since the one sex-chromosome carried by each male came from his mother, who was wild. On this hypothesis, we should expect no abnormal males from the cross F_1 normal male by wild female (table 3, Series b). From two of the three bottles of this cross, no abnormal males hatched. However, 18 abnormalities, out of a total of 224 flies, hatched from the third bottle. It will be noticed, also, that abnormal males appeared in two of the eight bottles (table 2, Crosses I and III) among the F_1 of the cross abnormal male by wild female (red or white). These cases were difficult to explain on the theory that the factor is sex-linked, and yet the other cases, where no abnormal males appeared, were difficult to explain on any other theory. The results thus far were perplexing.

The appearance of the small number of abnormal males in the above experiment, where no abnormal males are expected, may be explained on Bridges' hypothesis of non-disjunction of the sex chromosomes (Jour. Exp. Zool., vol. 15, no. 4). On this view, the two sex-chromosomes sometimes stick together and either remain in the egg when the polar bodies are formed, or else both are eliminated. Should an egg from which both of the sex-chromosomes have been removed be fertilized by a female-producing spermatozoan from the abnormal male—a spermatozoan that carries the sex-chromosome—an abnormal male would be produced. If abnormal males should appear frequently in the F_1 when the father only is abnormal, one would not be justified in applying this hypothesis, for non-disjunction is a

rather rare occurrence. I therefore repeated the preceding cross (abnormal male by wild female) using both normal (wild) and normal white eyed females. No abnormalities appeared among the 539 males in the F_1 (see table 4, Cross I). Each fly was carefully examined twice to eliminate possible error. To leave no doubt whatever, the F_1 normal males were bred in pairs to wild females (table 4, Cross II). No abnormal flies, male or

TABLE 4

Cross I

<i>P</i> Abnormal ♂ by wild red ♀					<i>P</i> Abnormal ♂ by normal white ♀					
F_1	Normal ♂.....	91	144	158	393	F_1	White normal ♂.35	55	56	146
	Normal ♀.....	104	173	154	431		Red normal ♀...58	58	66	182

Cross II

F_1 Normal ♂ (from Cross I) by wild ♀					Total
F_2 Normal (♂ and ♀).....	1474	751	635	355	3215

Cross III

F_2 Normal ♂ (from Cross II) by F_2 normal ♀					Total
F_3 Normal (♂ and ♀).....	375	332	942	254	1903

Cross IV

F_1 Normal ♀ (from Cross I) by wild ♂				Total
F_2	Normal ♂.....	172	176	348
	Normal ♀.....	211	282	493
	Abnormal ♂... 16	24	40	

female, appeared in the F_2 , and, when these were inbred, no abnormalities were found in the F_3 (table 4, Cross III). In this experiment non-disjunction males did not appear, for none of the F_1 males had received a chromosome containing the factor for abnormality. The F_1 females, on the other hand, when bred to wild males, produced some abnormal F_2 males (table 4, Cross IV), thus revealing clearly the essential difference between the inheritance of sons and daughters. Since an abnormal male can

transmit the factor for abnormality to his daughters only (except in rare cases, which may be explained by non-disjunction) the factor must be sex-linked. This conclusion was justified by subsequent work.

5. Females, homozygous for abnormal, may be normal

It has been shown that one dose of the factor A does not always produced abnormality. Even a double dose may not cause any abnormality, as is shown in the following experiment. Two abnormal males were bred separately to two wild females. The F₁ females, which were heterozygous, were then crossed to abnormal males. Half of the F₂ females were therefore homozygous, and half heterozygous for abnormality. The results are given in table 5.

TABLE 5

P Abnormal ♂ by wild ♀

F₁ ♀ (from above cross) by abnormal male

				Total
F ₂	{ Normal ♂.....	84	56	140
	{ Normal ♀.....	73	68	141
	{ Abnormal ♂.....	1	6	7
	{ Abnormal ♀.....	1	0	1

Thus only one of the females showed any abnormality, though every one carried at least one dose of the factor. Eighteen of the normal F₂ females were bred in pairs to wild males, with the following result:

F₂ Normal ♀ (from above) by wild ♂

F ₃	{	Normal ♂.....	21	4	9	68	6	16	26	13	3	19	42	27	32	3				
	{	Normal ♀.....	56	94	50	118	64	35	69	63	69	18	111	66	126	49	57	58	5	177
	{	Abnormal ♂.....	1	4	1	2	12	8	2	18	1	23	4	1	1	23	3	4		
	{	Abnormal ♀.....																		2

Sixteen of the normal F₂ females thus produced abnormal males. Only a small number of males hatched, indicating that the factor for abnormality may act as a semi-lethal factor. In four of the eighteen bottles, all the males which hatched were

abnormal. In these four cases, at least, the F_2 female must have been homozygous, as otherwise some of her sons would have been normal. It is thus evident that a female homozygous for abnormal may be normal.

These results throw light on the following tests of the normal and abnormal progeny of abnormal parents (table 6). Abnormal males and females were bred in pairs. F_1 normal males from this cross were bred to F_1 normal females, and F_1 abnormal males to F_1 abnormal females. Many of the F_1 abnormalities either died

TABLE 6

P Abnormal ♂ by abnormal ♀								Total	
Normal ♂	8	7	37	52					
Normal ♀	88	45	96	229					
Abnormal ♂	40	29	45	114					
Abnormal ♀	21	34	43	98					
F ₁ Abnormal ♂ by F ₁ abnormal ♀									
Normal ♂	6	1	7						
Normal ♀	33	35	68						
Abnormal ♂	23	3	26						
Abnormal ♀	4	18	22						
F ₁ Normal ♂ by F ₁ normal ♀									
Normal ♂	20	17	15	49	53	84	54	62	354
Normal ♀	52	74	38	80	96	130	103	99	672
Abnormal ♂	21	12	25	38	24	19	28	32	199
Abnormal ♀	3	3	28	8	8	8	17	18	93

or were infertile, but those that were fertile produced in F_2 39 per cent of abnormal individuals. The F_1 normals of the same parentage produced, when inbred, only 22.1 per cent of abnormal individuals. Abnormal offspring of abnormal stock thus yield more abnormalities than do normal offspring. The reason for this becomes apparent when we consider that the stock had not then been proven to be pure. Among the parent abnormalities were therefore probably both homozygous and heterozygous individuals, and when the F_1 were chosen, some of the normals among them may have been true normals, whereas the F_1 abnormalities chosen were at least heterozygous for A.

The proportion of abnormal flies in the stock bottles varied from time to time. Records of the stock showed that it might yield a high per cent one month and a low per cent the following month. Also, the flies in any one stock were of varied types. Some were only slightly abnormal, and some were so abnormal that they could scarcely walk. The slightly abnormal produced progeny as abnormal as that of the very abnormal. This was shown by the following tests, *a* and *b*.

Test a. A slightly abnormal male was mated to a slightly abnormal female, and a very abnormal male to a very abnormal female:

P ₁ Slightly abnormal by slightly abnormal		P ₁ Very abnormal by very abnormal	
F ₁	{ Normal.....	18	F ₁ { Normal.....28
	{ Abnormal.....	5	

The abnormalities in the two cases were similar;

From slightly abnormal parents		From very abnormal parents	
4 flies showing reduplication in 1st left leg		3 flies showing reduplication in 1st left leg	
1 fly showing reduplication in 3d right leg		2 flies showing reduplication in 1st right leg	

Test b. A stock which was yielding only a small percentage of abnormals was divided into two parts. The least abnormals were inbred and the most abnormals were inbred:

	Normal	Abnormal	Per cent of abnormal
From the most abnormal F ₁	386	110	22.2
From the least abnormal F ₁	400	119	22.9

The degree of abnormality and the per cent of abnormal offspring were thus approximately the same, whether the parents were very abnormal or only slightly so.

INFLUENCE OF THE ENVIRONMENT ON THE DOMINANCE OF ABNORMALITY

1. *The changing dominance at least partly dependent upon the temperature*

As has been said, the proportion of abnormal flies in the stock showed considerable variation, and on two occasions the abnormalities almost disappeared. In the summer of 1912, shortly after the appearance of the mutation, an apparent 'reversion' to the normal type took place, and only a few abnormal hatched. After careful selection and inbreeding of these, the abnormalities in the stock returned. Again, in the spring of 1913, as the warm weather came on, a similar 'reversion' took place, though some

TABLE 7

DEVELOPED AT REDUCED TEMPERATURE				DEVELOPED AT ROOM TEMPERATURE			
	Normal	Abnormal	Percentage of abnormal		Normal	Abnormal	Percentage of abnormal
Stock a....	46	44	48.8	Stock a....	235	43	15.4
Stock b....	33	71	68.2	Stock b....	90	11	10.8
Stock c....	198	87	30.5				

abnormal flies were found in all the bottles. Stocks which had been yielding a fairly large per cent of abnormal flies now yielded only a few. Since in both cases the 'reversion' took place at the approach of warm weather, it seemed possible that temperature affected the production of the abnormal flies. To test this assumption, mass cultures from several stocks were placed in an ice chest and kept there until the pupae had developed, when they were removed to room temperature. The result was decisive. The per cent of abnormal flies was from three to six times that of the controls which were taken from the same stocks and kept at room temperature (table 7).

Many things obscure up to this time became clear. For example, the fact that a male carrying the factor for abnormality or a female carrying one or two doses of this factor might be either

abnormal or normal, became obvious when the effect of the environment was taken into consideration. The unsuccessful attempts to obtain pure stock were evidently due to unfavorable environmental conditions. If this were the case, the stock, which had been selected for many generations, was most probably pure.

Pure stock was readily obtained by inbreeding pairs of the F_1 abnormals of abnormal parents and allowing the F_2 to develop in the ice box. The best record so obtained was as follows:

F_2	{ Normal ♂.....	.47
	{ Normal ♀.....	.80
	{ Abnormal ♂.....	.90
	{ Abnormal ♀.....	.67

The F_1 female used must have been homoyzous, for otherwise the per cent of abnormal males would not have been over 50 per cent. The pure stock thus obtained was used as a basis for all the remainder of the experimental work.

Many of the iced flies showed a greater degree of abnormality than any of those which had been previously studied. In many cases the legs were mere stumps and the flies could not hatch normally. When the pupa cases opened, the flies were drawn out of them with needles and these flies, almost without exception, proved to be very abnormal and incapable of locomotion.

Up to this point only the general influence of the cold was understood. The bottles put on ice had been kept there until the pupae were well formed. The following questions arose: (1) At what stage in the development must the cold be applied? (2) How long must the developing flies be left in the ice box in order to become abnormal?

2. Low temperature more effective if the developing flies are cooled at early stages

To answer these two questions, two series of experiments were undertaken. For both of these pure stock only was used. In the first experiment, pairs of the pure stock were mated, and in most cases, allowed to remain in the bottles for only one day.

This was done so that the eggs in one bottle would be approximately the same age. In some cases, the parents remained in the bottles two, three and four days. In all cases the average time of the egg-laying was considered as midway between the time when the parents were placed in the bottle and the time when they were taken from the bottle. The number of eggs was not counted. After the parents had been removed, the bottles were kept at room temperature and then put on ice at stated periods. Some were put in the cold immediately, and others were kept at room temperature from one to nine days before they were placed in the cold. After the eggs or larvae had been put into the ice chest, they were not removed, except to add food, until

TABLE 8

Age of eggs or larvae when cooled	Total	Per cent of	
		Abnormal	abnormal
$\frac{1}{2}$ day.....	12	12	100.0
$1\frac{1}{2}$ to 2.....	506	341	67.4
$2\frac{1}{2}$ to 3.....	290	148	51.0
$3\frac{1}{2}$ to 4.....	417	98	23.5
$4\frac{1}{2}$ to 5.....	699	80	11.4
$5\frac{1}{2}$ to 6.....	752	79	10.5
$6\frac{1}{2}$ to 7.....	510	43	8.4
$7\frac{1}{2}$ to 8.....	960	116	12.1
$8\frac{1}{2}$ to 9.....	306	33	10.8
9 to $9\frac{1}{2}$	393	15	3.8
Control, not cooled.....	1711	174	10.1

they had hatched. The temperature of the ice chest was not kept constant, but varied slightly about 10°C . Controls of the same pure stock were kept at room temperature. In these bottles, the flies emerged in about twelve days. The percentage of abnormal flies which hatched from the iced bottles was found to depend upon the stage at which the cold was first applied. The results are given in table 8. In those bottles which were put on ice immediately after the mating, many of the flies were infertile, so that the number of F_1 flies which hatched was very small. However, these were all abnormal. The longer the flies developed at room temperature, or, in other words, the longer the time before they were placed on ice, the smaller was the number of abnormal flies. The bottles kept in the room six

days or more than six days developed no more abnormal flies than those kept for the entire time at room temperature. When the larvae were left in the room nine to nine and one-half days, the percentage of abnormal flies fell to 3.8 per cent, but this evidently is not significant, as the total number of flies was small. The small number of abnormals in this case may have been due to an increased room temperature at the time these flies were developing. The results are plotted in diagram 2. The curve

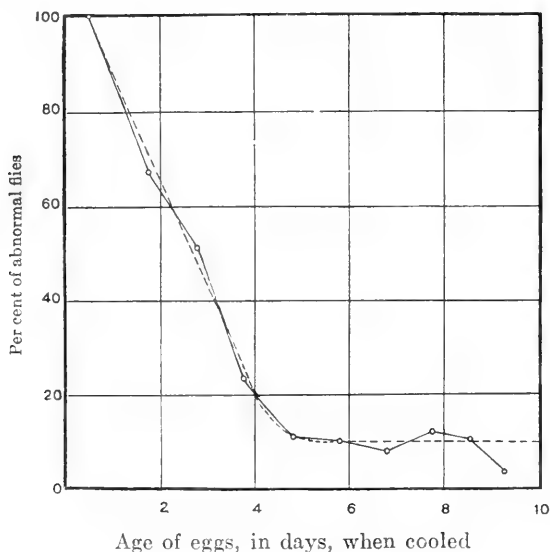


Diagram 2¹

of abnormality shows a rapid fall from its highest point, which represents the number of abnormals when the entire development took place at the low temperature, to a point corresponding to the number of abnormals when the first six days of development took place at room temperature. From this point the curve is nearly parallel to the abscissa. This indicates that low temperature is effective only if first applied at early stages, and that, after the larvae have developed a few days at room temperature, a decrease in temperature is ineffectual in causing abnormalities.

¹ Dotted line is theoretical curve.

3. Low temperature more effective if maintained throughout development

In the second experiment, eggs were put on ice immediately and kept there for different lengths of time, after which they were removed to room temperature for the remainder of their development. Flies of pure stock were placed in bottles containing banana spread out on narrow strips of paper. The food was removed at short intervals, one hour, two hours, etc., though in some cases it remained in the bottles over night. As soon as removed, the food was carefully examined and the eggs were picked from it, one by one, by the aid of a flattened needle, and placed on fresh banana in a clean bottle. Their number was

TABLE 9

DAYS ON ICE	NO. OF EGGS	NO. EGGS HATCHED	PER CENT HATCHED	NORMAL	ABNORMAL	SUM	PER CENT OF ABNORMAL
1 to 5.....	1370	644	47.0	589	44	633	7.0
6 to 10.....	608	290	47.7	209	77	286	26.9
11 to 16.....	260	99	38.1	40	50	90	55.6
22 to 24.....	46	17	37.0	4	8	12	66.6
32 to 35.....	44	13	29.6	2	9	11	81.8

recorded. When the eggs were transferred, they were not placed directly on the banana but upon a piece of filter paper placed over the food. This was done to avoid covering the eggs with the food, which may prevent them from hatching. The bottles were then placed on ice. Some were kept there one to five days, others six to ten days, etc. The bottles were then removed to room temperature. Table 9 and diagram 3 give the results. Some of the flies which hatched had lost one or more legs and therefore could not be classed as abnormal or normal. For this reason the 'number hatched' is larger than the sum of abnormal and normal.

The per cent of abnormal flies varied directly with the length of exposure to cold, but the number hatched varied inversely with the length of exposure to cold. As many of those which did

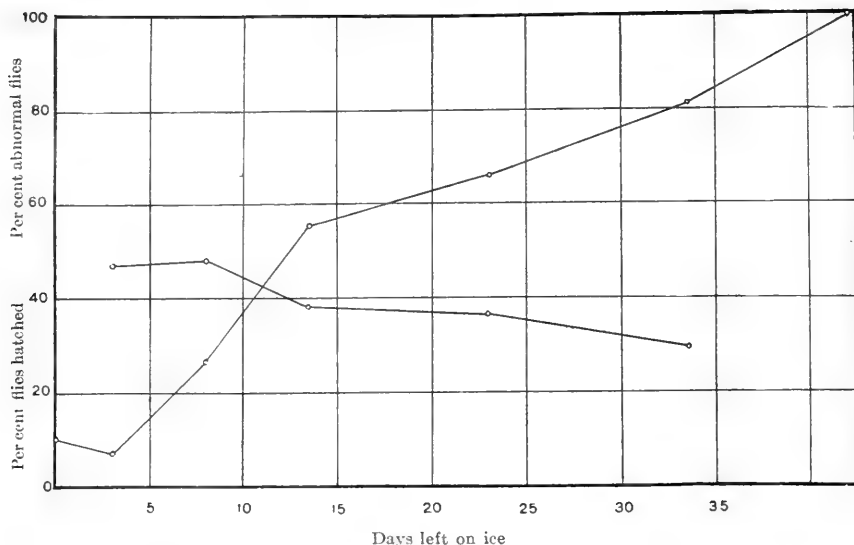


Diagram 3

not hatch would have been abnormal, the percentage of abnormal flies would probably have been larger, had the full number hatched.

These two experiments show conclusively that a temperature of $10^{\circ}\text{C}.$ \pm , maintained throughout development, produces a large percentage of abnormal flies.

LOW TEMPERATURE WITHOUT EFFECT ON WILD STOCKS

Flies from a wild stock were mated, both in pairs and in mass culture and placed in the ice box immediately or several days after mating. The larvae were removed before they hatched. Of the 1352 flies which hatched, only one showed any abnormality. The test was repeated, and this time the eggs were cooled shortly after they were laid, and the pupae were allowed to hatch in the ice box. The 214 flies which hatched were all normal. Low temperature, therefore, does not cause reduplication in normal stock. The one abnormal fly that appeared in the first case was probably due to infection. Possibly it may have been a mutant. On the other hand, the abnormality may

have been simply a somatic variation, for a few abnormal flies, similar to those of my stock, have been found from time to time in other cultures, but when bred, they have never transmitted the variation.

SELECTION AND MUTATION

1. Repetition of selection of wild stocks not followed by mutation

As the abnormal flies originally appeared in a selection experiment, it might seem probable that selection had some influence on the appearance of the mutation. Were such the case, the mutation should appear again if the original selection should be repeated. Wild stock was therefore selected, as before, for a high number of teeth in the sex-comb. The selection was carried on even longer than in the first case, but no abnormal flies were discovered.

2. Selection in the reverse direction also not followed by mutation

Selection for the smallest number of teeth in the sex-comb was carried out. From wild stock a number of flies having eight or nine teeth were mated in pairs to wild females. From the F_1 and succeeding generations the males that had the smallest number of teeth were selected for further mating. Not all the males were examined in each generation, but in the sixth and tenth generations careful counts were made.

<i>6th generation</i>		<i>10th generation</i>	
a.....	9.11	a.....	9.30
b.....	8.89	b.....	9.40
c.....	8.90	c.....	9.40
d.....	10.30	d.....	9.10
Average.....	9.30	e.....	9.20
		f.....	8.20
		Average.....	9.15

The average number of teeth in the wild stock is ten. Selection downward has brought about a decrease in this number. The flies which hatched in each generation of both this and the previous selections were carefully examined for leg abnormalities, but no mutations in the legs were found.

As a check on the selection experiments, wild stocks from various localities were examined, and the average number of teeth of 30 to 40 individuals in each stock thus obtained:

Seattle, Washington.....	9.40
Liverpool, Nova Scotia.....	10.60
Harris, Minnesota.....	9.96
Newport, California.....	9.50
Madison, Wisconsin.....	10.20
Sheridan, Wyoming.....	10.40
Santiago, Cuba.....	10.50
Guantanamo, Cuba.....	10.60
Average of averages.....	10.14

A slight variation thus appears in different stocks, but this may be due to the small number counted.

THE LINKAGE BETWEEN THE FACTOR FOR REDUPLICATION AND OTHER SEX-LINKED FACTORS

Red-eyed abnormal males were crossed to normal females with white eyes or barred eyes or vermilion eyes. These three characters are sex-linked. The first generation was allowed to develop at room temperature. The flies were then mated at room temperature, and later transferred to new bottles and placed in the ice chest, where the eggs were laid. Mating at room temperature was found to be necessary, as it evidently does not take place at the low temperature. The eggs developed very slowly, and, although many reached the late larval, or early pupal stage, development did not proceed farther in most cases. A few flies emerged at the low temperature, but the numbers were too small to be significant. The same matings were then repeated, but with some changes in method. When the F_1 hatched, they were mated both in pairs and in mass cultures, and then removed not to an ice box, but to a cold room, the temperature of which varied approximately from 10 to 15°C. After the F_2 larvae had pupated (about 20-28 days after mating of F_1) they were removed to the normal room temperature, at which the flies hatched. No attempt was made, as in some of the previous experiments (tables 8 and 9), to have the conditions nearly identical for all the developing flies, as the experiment was planned to obtain

as many abnormals in F_2 as possible. Thus, when the bottles were removed to room temperature, although some of the larvae had pupated, others were still in the larval stage. By this method a much larger yield was obtained, and a fair percentage of those which hatched were abnormal.

Two kinds of controls were used. In the first, the F_1 flies which had laid in the cold room were transferred to new bottles, and a second batch of eggs was laid at normal room temperature, where the entire development took place. For the second control, abnormal flies from pure stock were mated, and kept in the cold room, as in the case of the F_1 described above. The expectation and the count are as follows:

CROSS I ²				
Vermilion normal ♀ $\left(\frac{v a' X}{v a' X}\right)$ by red abnormal ♂ $\left(\frac{VA' X}{0}\right)$				
F_1 red ♀ heterozygous $\left(\frac{v a' X}{VA' X}\right)$ and vermillion normal ♂ $\left(\frac{v a' X}{0}\right)$				
	Non-cross-over eggs	Cross-over eggs	Sperm	
Gametes of F_1	$v a' X$ $V A' X$	$v A' X$ $V a' X$	$v a' X$ 0	
F_2 (expectation)	Vermilion normal ♀ $\left.\frac{v a' X}{v a' X}\right\}$	non-cross-overs	vermillion abnormal ♀ $\frac{v A' X}{v a' X}$	cross-overs
	Red abnormal ♀ $\frac{V A' X}{v a' X}$		red normal ♀ $\frac{V a' X}{v a' X}$	
	Vermilion normal ♂ $\frac{v a' X}{0}$		vermillion abnormal ♂ $\frac{v A' X}{0}$	
	Red abnormal ♂ $\frac{V A' X}{0}$		red normal ♂ $\frac{V a' X}{0}$	

² The symbols used in the text are as follows: Br' , dominant factor causing barred eyes; br' , its allelomorph in the wild red fly; w , recessive factor causing white eyes; W , its allelomorph in the wild red fly; v , recessive factor causing vermillion eyes; V , its allelomorph in the wild red fly; A' , dominant factor causing abnormal legs; a' , its allelomorph in the wild fly. In Crosses I and II, the F_1 should show complete criss-cross inheritance, the females being like their father and the males like their mother, since the female parent carried only recessive sex-linked characters. Practically all the F_1 in all three crosses were normal, since they had developed at room temperature.

		(Control 1)	
Developed at low temperature (22 bottles)		Developed at room temperature (18 bottles)	
F ₂ (count)	Normal	red ♀.....	1712
		vermilion ♀..	1908
		red ♂.....	329
		vermilion ♂...	1654
	Abnormal	red ♀.....	3
		red ♂.....	530
vermilion ♂...		5	
Total number of non-cross-over abnormal males ³		656	
Total number of cross-over abnormal males.....		11	
Total number of F ₂ abnormal males.....		667	
Ratio of cross-overs to total number.....		1.6 per cent	

CROSS II

White normal ♀ $\left(\frac{w a' X}{w a' X}\right)$ by red abnormal ♂ $\left(\frac{W A' X}{0}\right)$

F₁ red ♀ heterozygous $\left(\frac{w a' X}{W A' X}\right)$ and white normal ♂ $\left(\frac{w a' X}{0}\right)$

		Non-cross-over	Cross-over		
		eggs	eggs	Sperm	
Gametes of F ₁		w a' X	w A' X	w a' X	
		W A' X	W a' X	0	
F ₂ (expecta- tion)	White normal ♀	$\frac{w a' X}{w a' X}$	non-cross-overs	white abnormal ♀	$\frac{w A' X}{w a' X}$
	Red abnormal ♀	$\frac{W A' X}{w a' X}$		red normal ♀	$\frac{W a' X}{w a' X}$
	White normal ♂	$\frac{w a' X}{0}$		white abnormal ♂	$\frac{w A' X}{0}$
	Red abnormal ♂	$\frac{W A' X}{0}$		red normal ♂	$\frac{W a' X}{0}$
				cross-overs	

Developed at low temperature (18 bottles)		(Control 1)	
		Developed at room temperature (5 bottles)	
F ₂ (count)	Normal {	red ♀.....969	352
		white ♀.....892	304
		red ♂.....346	298
		white ♂.....579	298
	Abnormal {	red ♂.....250	47
		white ♂..... 90	31

³ In calculating the linkage, only the F₂ abnormal males are considered. All the F₂ abnormal males, whether developed in the cold room or at normal room

Total number of non-cross-over abnormal males.....	297
Total number of cross-over abnormal males.....	121
Total number of F ₂ abnormal males.....	418
Ratio of cross-overs to total number.....	28.9 per cent

CROSS III⁴

Barred normal ♀ $\left(\frac{\text{Br}'a'X}{\text{Br}'a'X} \right)$ by not-barred abnormal ♂ $\left(\frac{\text{br}'A'X}{0} \right)$

F₁ barred ♀ heterozygous $\left(\frac{\text{Br}'a'X}{\text{br}'A'X} \right)$ and barred normal ♂ $\left(\frac{\text{Br}'a'X}{0} \right)$

		Non-cross-over eggs	Cross-over eggs	Sperm
Gametes of F ₁		Br'a'X br'A'X	Br'A'X br'a'X	Br'a'X 0
F ₂ (expecta- tion)	Barred normal ♀	$\frac{\text{Br}'a'X}{\text{Br}'a'X}$	barred abnormal ♀	$\frac{\text{Br}'A'X}{\text{Br}'a'X}$
	Barred abnormal ♀	$\frac{\text{br}'A'X}{\text{Br}'a'X}$	barred normal ♀	$\frac{\text{br}'a'X}{\text{Br}'a'X}$
	Barred normal ♂	$\frac{\text{Br}'a'X}{0}$	barred abnormal ♂	$\frac{\text{Br}'A'X}{0}$
	Not-barred abnormal ♂	$\frac{\text{br}'A'X}{0}$	not-barred normal ♂	$\frac{\text{br}'a'X}{0}$
		non-cross-overs		cross-overs

Developed at low temperature (22 bottles)		Control 1 Developed at room temperature (8 bottles)
F ₂ (count)	Normal	1433
	barred ♀.....	598
	barred ♂.....	381
	not-barred ♂..	167
	Abnormal	46
	not-barred ♂..	
	barred ♂.....	

Total number of non-cross-over abnormal males.....	463
Total number of cross-over abnormal males.....	120
Total number of F ₂ abnormal males.....	583
Ratio of cross-overs to total number.....	20.6 per cent

temperature, are counted together, as the F₁ parents were identical for both. The bottles were carefully examined, and when the pupa cases opened, the flies, if unable to emerge, were drawn out with needles. Many of the abnormal males were thus artificially hatched, and it was only by this method that so large a yield was obtained.

⁴ In Cross III, the F₁ should show criss-cross inheritance with respect to factor A only, as the factor for barred is dominant in the female parent.

In crosses like Cross III, where one or both dominant sex-linked characters entered with the female parent, the 'cross-overs' are recognizable only in the male class, since all the F_2 females carry the factor which was dominant in their grandmother. In crosses where both sex-linked characters are recessive in the female, as in Crosses I and II, the F_2 females, as well as males, can usually be used in determining the linkage. In the present experiment, however, the female 'cross-overs' must necessarily be disregarded, since, as will be shown later, female flies carrying A are more often normal than are males carrying A.

It is obvious that all of the F_2 males can not be used to determine the linkage, since some males that are normal, whether barred, vermilion or white, may carry the A factor. The cal-

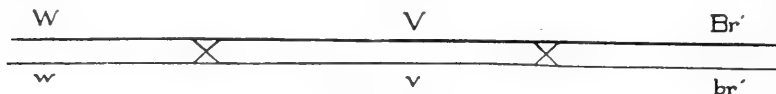


Diagram 4

culation of the linkage is, therefore, made on the following basis. Though all of the F_2 flies are recorded, the abnormal males only are considered in determining the linkage. The ratio between the abnormal 'cross-over' males and the total number of abnormal males is thus regarded as a measure of the distance between the A factor and the other factor under consideration. This scheme rests upon the assumption that barred, vermilion, white, and red flies, carrying A, develop the reduplications to an equal extent. This assumption seems justifiable, as there is no evidence that any interaction takes place between the A and other factors.

The results given above show that the linkage between the A factor and the three other factors compared above is strongest in the case of vermilion (1.6), much weaker with barred (20.6), and still weaker with white (28.9). Sturtevant ('13) showed that 30.7 per cent of crossing-over occurs between white and vermilion, and Tice ('14) showed that 25.3 per cent of crossing-over took place between barred and vermilion. These three factors may be diagrammatically represented as in diagram 4. The strength

of the linkage between vermilion and abnormal indicates that their loci are close together. This conclusion is further supported by the linkage between white and abnormal (28.9) and between barred and abnormal (20.6). These distances are reasonably close to those obtained by Sturtevant, for white and vermilion, and by Tice, for barred and vermilion. Recent work done in this laboratory has shown that a double crossing-over, involving two breaks in the chromosome, may take place when long distances are concerned. When this occurs, the non-cross-over classes are increased and the cross-over decreased. Allowing for a larger number of double cross-overs in the present work than in that of Sturtevant and Tice, the results fall within the limits of variation which might be expected if vermilion were as close to abnormal as the per cent of cross-overs (1.6) indicates. It seems hardly necessary to determine on which side of vermilion the A factor is located, since the nature of the character will necessarily exclude it from other linkage experiments.

These results afford additional evidence, were such necessary, of the location of the A factor in the sex-chromosome and of the identical behavior of this and other sex-linked factors.

THE FACTOR FOR REDUPLICATION A SEMI-LETHAL

A second control of the above crosses was made by allowing flies of pure abnormal stock to develop at the low temperature until the pupa stage had been reached, when they were removed to normal room temperature. The feature which distinguished this control from the main test was thus not a difference in the environmental conditions, as in the first control, but a difference in the genetic constitution of the flies. The following flies hatched from this control.

		P—Abnormal stock (5 bottles)	
F ₁	{	Normal ♀	391
		Normal ♂	105
		Abnormal ♀	151
		Abnormal ♂	301

It was necessary to hatch many of these flies artificially, as before, but the normal individuals were perfectly normal. There-

fore, the low temperatures maintained during the main test were either kept too high or not continued long enough to influence all the flies carrying the A factor, but only by this method could a sufficient number of abnormal flies be obtained. It is important to note that from the low temperature bottles of the abnormal stock, a smaller number of males than females was hatched, and that the larger proportion of the abnormals were males. Thus the low temperature, which has been shown in table 9 to exert a lethal effect on pure abnormal stock, is more fatal to males than to females.

Previous experiments (table 9) have shown that, when pure abnormal stocks develop at low temperature, the per cent of abnormals is increased, but the total number of flies decreased. Abnormal flies are thus much less viable than are flies of wild stocks. However, as the abnormals appear more frequently when the larvae are developed at reduced temperature, the low viability of this stock is much more pronounced under these conditions. In crosses with the wild, when the F_2 flies have developed at room temperature, those classes which, in the absence of cross-over, carry the factor for abnormality, as the red-eyed flies in Crosses I and II and the not-barred flies in Cross III fall only slightly, if at all, below the other classes. If the F_2 flies are raised at reduced temperature, however, these classes fall far below the others. This decreased viability is often noticed in the males alone, for, as stated above, females, even when homozygous for A, are less affected by a low temperature. Upon the males, the factor for abnormality has a definite lethal effect, which is greatly increased by reduced temperature. This fact is well shown by the following figures, in which the F_2 recorded above are grouped according to eye character alone:

CROSS I

Vermilion normal ♀ by red abnormal ♂

	Developed at low temperature	Developed at room temperature
Red ♀.....	1715	980
Vermilion ♀.....	1908	1016
Red ♂.....	859	737
Vermilion ♂.....	1659	884

CROSS II

White normal ♀ by red abnormal ♂

	Low temperature	Room temperature
Red ♀	969	352
White ♀	892	304
Red ♂	596	345
White ♂	666	329

CROSS III

Barred normal ♀ by not-barred abnormal ♂

	Low temperature	Room temperature
Barred ♀	2536	1433
Not-barred ♂	674	548
Barred ♂	1020	644

These figures show that the lethal effect is also evident in those classes of males which would not, in the absence of cross-over, carry the factor for abnormality. In Crosses I and II, were there no lethal effect, all four classes should be equal, and in Cross III the number of not-barred and barred males should in each case be one-half that of the barred females. Not only are the red males in Cross I and II and the not-barred males in Cross III far below the other classes, but the vermilion, white and barred males are also low in number. The decreased viability of these groups is directly proportional to the extent to which the vermilion, white and barred factors cross-over with the factor for the normal red-eye. Thus, in Cross I, the number of the vermilion males was only slightly less than that of the red or vermilion females, for only seldom (1.6) does 'crossing-over' affect the vermilion males. In Crosses II and III, the white and barred males showed a larger decrease than did the vermilion males, since the linkage was stronger between abnormal and vermilion than between abnormal and either barred or white. Also, the white males were more affected than the barred males, as 'crossing-over' took place oftener in the case of white (28.9) than in the case of barred (20.6).

It is interesting to contrast the results given above with those obtained by Rawls and by Morgan. The former discovered among her cultures an unusual sex-ratio of two females to one male. Morgan found that this ratio was due to a sex-linked

factor, one dose of which is fatal to the male. A female homozygous for lethal can never be produced, but a heterozygous female with one dose can survive. In the case of the reduplicating factor A, a single dose may be more or less fatal, depending partly on the environmental conditions during the larval stages. It is evident that the lethal effect was greater when development took place at reduced temperature. This was especially apparent when the first flies began to hatch from the cooled bottles in the crosses given above. Since the eggs had not all been laid at the same time, and since the bottles had been removed to a warm room when the larvae began to pupate, the first flies to hatch were necessarily those which had been longest under the influence of the cold. In the case of a number of bottles, several days elapsed after the first hatching before any red or unbarred males appeared. Vermilion, white or barred males hatched almost from the start, so that the delay in hatching applied to the one male non-'cross-over' class which carried the A factor. This result is entirely in harmony with that given in table 9 and shows that the lethal effect of the A factor varies directly in proportion to the length of exposure to cold. This factor therefore acts as a semi-lethal. If many of the flies in the above experiment had not been artificially hatched, the lethal effect would have been still more pronounced. As practically all the flies which reached the pupa stage were included in the count, it seems probable that the A factor in many cases exerts its fatal influence during the early stages of development.

SYMMETRY OF REDUPLICATED PARTS

1. *In Drosophila*

The abnormal legs present a great variety of types, a number of which are here figured. A detailed description of the legs is given with the figures. Special attention has been paid to the symmetry of the extra parts, and its relation to the symmetry of the normal leg. The symmetry is estimated from the manner in which the parts are bent upon each other and from the position of the claws on the last tarsal segment. In the case of the

first pair of legs of the male, the position of the sex-comb has also proved a reliable guide. If the sex-comb is lacking, or its position uncertain, the symmetry of the first leg is determined by the claws, and the reverse applies when the claws are lacking. If a leg has two parts, that are right and left, the symmetry is indicated as R, L, the part to the left of the figure being first indicated. Two such parts are designated as secondary. If one of two such secondary parts be again bifurcated into two so-called tertiary parts, the symmetry of the whole is indicated by separating the letters which represent the two secondary parts by a semicolon, thus—R; L, R. The letters X, Y, and Z are used when the symmetry can not be determined. When a leg has three parts, all of which seem to originate at the same level, the three letters representing these parts are separated by commas, thus—R, L, R. When there are more than three parts originating from one leg, colons and then periods are used to separate the letters representing the parts.

A comparison of the legs figured and described in the plates shows that they readily fall into a number of definite types, as follows:

Type a. Simple bifurcation occurring at any level of the leg from coxa to tarsus, and involving many or only a few segments. The two parts so formed may usually be identified as left and right respectively; i.e., they may be considered as mirror images of each other, though one of the two parts is sometimes larger than the other. Examples:

Bifurcation at trochanter, figure 30, second left leg, R, L
Bifurcation at femur, figure 14, left leg, L, R(?)
Bifurcation at femur, figure 19, right leg, R, L
Bifurcation at femur, figure 20, right leg, R, L
Bifurcation at femur, figure 22, left leg, R, L
Bifurcation at femur, figure 38, left leg, R, L
Bifurcation at tibia, figure 35, right leg, X, Y

Figures 5, 8 and 34 may be of this type, but the legs are too incomplete to determine this definitely.

Type b. Bifurcation into two secondary parts and further division of one of the secondary parts into two tertiary parts. This

is a very common type. Usually the two tertiary parts may be identified as left and right with respect to each other and that tertiary part that is nearer the undivided secondary is a mirror image of it. Moreover, the undivided secondary, practically without exception, retains the symmetry of the normal leg. Thus if the whole leg be a right, the undivided secondary is a right. Examples:

First division at coxa, left 1st leg, figure 24, L; R, L
 First division at femur, right 1st leg, figure 23, R; L, R
 First division at femur, right 1st leg, figure 18, R; L, R
 First division at femur, right 1st leg, figure 16, R; L, R
 First division at tibia, left 1st leg, figure 11, L, R; L
 First division at tibia, left 1st leg, figure 13, L; R, L
 First division at tibia, right 1st leg, figure 15, R; L, R
 First division at tibia, left 2d leg, figure 31, L, R; L
 First division at tarsus, right 1st leg, figure 10, R, L?; R
 First division at tarsus, left 1st leg, figure 12, L; X, Y

Type c. In examples of this type, there is at most one division of segments, and often the leg is undivided, but two divisions are indicated by the number of claws or sex-combs. Examples: (a) No division of segments, but tarsus with 2 combs and enlarged distal segment having 3 pair of claws, two of which have a common pad. Left first leg, figure 2, L; R, L. (b) A single division of segments resulting in two tarsi, one of which bears three claws. Right first leg, figure 7. (c) A single division of segments resulting in two tarsi, one of which has two sex-combs and probably always two pairs of claws: Division at femur, left first, figure 15, L, R; L; Division at femur, left third, figure 37, L, R; L; Division at tarsus, right first, figure 6, R; L, R; Division at tibia, left first, figure 9, L, R; L.

Type d. In examples of this type, three parts proceed from a single undivided segment. I have only two undisputed cases of this type. In one of the two specimens, the outer and inner parts have the same symmetry and they are mirror images of the middle part between them. The symmetry cannot be determined in the other specimen, as the claws are lacking. Examples: Division at first tarsal segment, left first, figure 4, L, R, L; Division at fourth tarsal segment, left first, figure 3.

Type e. After a single division, resulting in the formation of two secondary parts, each of the latter shows one or more further divisions, involving segments or only parts of segments. Examples: (a) First division at femur and second division involving tarsus of each part. Right first, figure 17, L, R; L, R. (b) First division at femur. One of the secondary parts divides into two, and the other into three tertiary parts. First division at the femur. Second division at tibia or tarsus, Left first, figure 19, R, X; L, R (?), L.

Type f. This type is simply a continuation of the process of division seen in Type b, and involves a division into two secondary parts, one of which remains single, whereas the other shows several further divisions. First division at femur, second division at tibia, and third and fourth divisions at tarsus. Left first, figure 21, R: X; Y, Z. L.

Type g. No division of segments occurs, but parts of abnormal size and shape. Examples: Right first, figure 26; left first, figure 27; left third, figure 32.

Type h. No division of parts, but complete fusion of the two legs of the opposite sides. Example, figure 25, which represents the fused left and right legs of the first pair. In figure 19, of Type e, division has occurred, but in this case, also, the first pair of legs is more or less completely fused.

All of the abnormal legs of *Drosophila* that I have studied belongs to one of these nine types. Many examples of nearly all the types have been observed, but drawings were made only when a new variety of the type was found. At first sight, these types may seem unconnected by any plan of division. Careful study, however, will reveal the fact that, although the first six types are dissimilar, the difference is one of degree, and not of kind. All have evidently been produced by one or more bifurcations. In Type a, only one division has taken place, and only two parts have resulted. The two parts are mirror images and there is no reason to assume that either of them is duplex in value. The examples of Type b obviously result from two successive bifurcations, the first, which is similar to the single division of Type a, and the second, which affects only one of the

parts formed by the first division. In Type c, the second division does not involve the division of segments and it is indicated only by the double claws or double combs. It is possible that in these cases, the claws and combs alone, and not the segments between them, should be regarded as duplex, but it seems more probable that such forms represent legs in which the second division is incomplete, or in which the parts formed by the second division have become more or less fused.

The symmetry of the three parts is very definitely regulated. In all examples of the two types—b and c—in which the symmetry of the three parts can be determined, the following relations are clear. The undivided secondary part formed by the first division maintains the true symmetry of the leg. If the leg be a right, the undivided secondary is a right, no matter whether it be the outermost or the innermost of these three parts. The tertiary part next to it is a mirror image of it and the tertiary part farther from it is like it in symmetry. The symmetry of the two tertiary parts appears as though regulated by the symmetry of the undivided secondary part. This conclusion is the only one possible if it be granted that Type b is only a further development of Type a.

The three apparently equal parts in the legs of Type d proceed from one undivided segment. This form of reduplication may seem very different from that just described; but I am inclined to think that it is simply a modification of Type b, in which both divisions have occurred at the same level of the leg.

If the process of division, characteristic of the types thus far described, should be further continued, it would lead to the formation of forms such as those described under Types e and f. In Type e, both instead of one of the secondary parts, formed as in Type a, divide, and if no further division occurs, a form like that of figure 17 results. It should again be pointed out, however, that a division of the sex-comb may or may not indicate a duplex nature of the part, though the former supposition seems more probable. If, however, there should be a further division, involving only one of the four tertiary parts, five parts, instead of four, would be formed. Four parts might also arise, not as in

figure 17, by simultaneous division of both secondary parts, but by a continuation of the process begun in Type b. Thus, if the undivided secondary should still remain simplex, and if one of the two tertiary parts, formed by division of the other secondary, should divide, four parts would result. I have no examples of this kind, but figure 21 involves the same process in which the division has proceeded one step further. This leg is particularly interesting, since it shows to what an extent the successive divisions may take place. The respective values of the parts in this leg are very clear.

The symmetry of these legs having four or five parts leads to some further considerations. Since in general, two parts with the same symmetry are not adjacent to each other it is probable that, with each subsequent division, the symmetry of the new parts is regulated by the symmetry of the adjacent parts. We may thus conclude, that a single division, as in Type a, results in the formation of a right and left, either of which may be on the outer side. The symmetry of a part formed by a further division of one of these two is determined, not by any special innate potentiality which it possesses, but by its position with respect to the adjacent parts.

When the abnormal flies developed at low temperature, the legs often showed a greater degree of abnormality than when development took place at normal room temperature. The basal parts were frequently very much enlarged, and sometimes divided, as in figure 36. In other specimens, no division of the segments had taken place (Type g, figs. 26, 27 and 32). Legs of this type were usually found on flies having other legs which were more or less divided, and as they have never, to my knowledge, been found on flies of the normal wild stock, it may safely be concluded that they, also, are due to the A factor carried by the abnormal flies. All the other types may be explained as due to one or more divisions, which in some cases were indicated only by double combs or by extra claws. Since the division may thus manifestly affect such small parts only, it seems reasonable to conclude that in the abnormally shaped legs of Type g, the divisions have affected only parts of the segments, or groups

of cells. The three groups of teeth linearly arranged in the first tarsal segment of figure 26, lends support to this view of general subdivision.

It seems probable that the cells of the leg anlage in those flies which carry the A factor are extremely plastic, easily divided, and as easily united when contact occurs.

The results of the study of the symmetry of abnormal legs may be briefly summarized as follows:

1. Extra parts may arise at any level of the leg.
2. The parts contained in the extra appendages are only those which are distal to the point of origin, and all the distal parts are in most cases represented. Figures 2 and 23 are exceptions to this and are probably due to a linear fusion of segments.
3. The reduplicated parts are the results of a continued bifurcation, and any part may continue to bifurcate although the other parts may show no further division. By four bifurcations one leg may thus form five parts.
4. When a single division takes place, the resulting parts are left and right respectively. When further division of one of the secondary parts takes place, the symmetry of the resulting tertiary parts bears a relation to that of the undivided secondary in that two parts of the same symmetry are never adjacent to each other. The undivided secondary retains the normal symmetry of the leg.
5. After a division, the resulting parts may be more or less fused, and the duplex nature indicated only by a double comb or extra claws. I consider a part as duplex only when some definite structure which is characteristic of that part is duplex, for otherwise one is forced to assume that the entire limb is duplex, whenever any distal part of it shows indication of doubling. Duplex parts, in fact, are usually preceded by proximal parts no larger than those of a normal leg.

2. Reduplication in other animals

Reduplication of the appendages has been found in nature among many groups of animals, but it has been most frequently observed in arthropods, amphibia, and mammals. The extra parts observed in arthropods usually consist of two supernumerary appendages. In mammals and in amphibia, many kinds of reduplication have been found, such as an extra pelvic girdle with two supernumerary limbs (amphibia); one or two extra extremities more or less complete (amphibia, birds, mammals, man); extra digits (cat, man, etc.). The extra parts are often somewhat incomplete or imperfect. In man, polydactyly is the most common form of reduplication. There are various kinds of polydactyly, and in the majority of cases, there is no manifest change in the symmetry of the hand or foot, and the extra digit is apparently a reduplication of one of the normal digits. A new symmetry is, however, sometimes established, as in double hands and feet.

Bateson's pioneer work on "The materials for the study of variation" has done much in bringing together examples of meristic phenomena from all the main phyla. In this work Bateson has described reduplication in insects, crustacea, birds and various mammals, artiodactyls, perissodactyls, cats and man. In his recent work on "Die Vertebraten Hypermelie," Pol has collected more evidence concerning the meristic variations of vertebrates, especially of man, and he has also included a summary of the experimental work on regeneration of limbs of amphibians.

3. Reduplication in insects

Bateson has formulated a number of rules to apply to reduplication in insects. The substance of these is as follows:⁵

1. The parts composing extra legs do not as a rule greatly differ from those of the normal legs which bear them.

2. The parts found in the extra legs are those parts which are in the normal leg peripheral to the point from which the extra legs arise, and as rule, no more.

⁵ Bateson, Materials for the study of variation, p. 476.

3. Extra legs may arise from any point on the normal leg, . . . but there is a slight preponderance of cases beginning from the apex of the tibia

4. It does not appear that extra legs arise more commonly from either of the three normal pairs in particular.

5. Supernumary legs of double structure are sometimes found as two limbs separate from each other nearly or quite from the point of origin, but in the majority of cases their central parts may be so compounded together that they seem to form but one limb, and the essential double character of the limb is not then conspicuous except in the periphery.

Rule I. The long axes of the normal appendage and of the two extra appendages are in one plane; of the two extra appendages one is therefore nearer to the axis of the normal appendage and the other is remoter from it.

Rule II. The nearer of the two extra appendages is in structure and position formed as the image of the normal appendage in a plane mirror placed between the normal appendage and the nearer one, at right angles to the plane of the three axes; and the remoter appendage is the image of the nearer in a plane mirror similarly placed between the two extra appendages.

Bateson states⁶ that "it is practically certain that in no case can a single, viz., an unpaired duplicate of the normal appendage grow from it." Thus, he says, "a limb of one side of the body, say the right, has in it the power to form a pair of limbs, right and left, as an outgrowth of itself, but cannot form a second left limb alone." He explains examples in which there is a single unpaired duplicate of the normal appendage as instances of a supernumerary pair in which only the two morphologically anterior or two morphologically posterior surfaces are developed.

If we compare these conclusions with those obtained in the present study of reduplication in *Drosophila*, we find that many of the observations and rules of Bateson which are given above are in general confirmed. I have found, however, a greater number of flies showing reduplication of the first pair of legs than of any other, though many flies with extra second or third legs have been observed.

The symmetrical relations which Bateson observed hold for *Drosophila* in those cases in which two extra parts have grown from the normal leg. The same relations also hold, in general,

⁶ Problems of genetics, p. 75.

for those legs of *Drosophila* in which there are more than two extra parts, although Bateson had no examples of this kind. Bateson's statement that a right limb can not form a second left limb alone appears arbitrary in the light of the present work, since, in a number of cases, a single unpaired extra part, a mirror image of the normal, has been observed. Since such single extra parts show no indication of doubling, I am not convinced that there is any advantage in regarding them as double. We have thus in *Drosophila*, legs with from one to four extra parts, the latter having certain definite symmetrical relations to each other and to the normal leg.

4. *Comparison between the extra appendages of Drosophila and other animals*

Bateson found no examples like those last mentioned, and, since he regarded all insect reduplications as composed of two extra parts grafted upon the normal, he was unable to homologize the variations of insects with those of mammals. If, however, the type which Bateson regarded as universal be considered as only one of a number of types, then the reduplication of both insects and mammals may be shown to result from a more or less continued process of division. If, with this suggestion in mind, the figures given by Bateson and Pol be again examined, many reduplications, morphologically very different, will probably be brought into line. It must be remembered, however, that some of the parts formed by the divisions may fail, as in *Drosophila*, to attain complete development. This is a possible explanation of some of the forms of polydactyly found in man.

5. *Comparison with the regeneration of experiment of Tornier*

The work of Tornier and Fritsch on regeneration has thrown much light on the origin and meaning of the reduplications found in nature. This work is briefly summarized by Pol. Fritsch cut off the hand of Triton, and then, following the method of Barfurth, he made a longitudinal cut in the fore limb. In two cases a hand was regenerated from each of the cut surfaces

at the end of the forearm. Tornier also performed similar experiments. In one case, he cut off the hind leg of Triton near the hip joint and stretched a thread across the cut stump, so that the femur was practically divided at the cut end. A double limb, with the two feet turned toward each other, regenerated from the cut femur. Tornier carried on a still more interesting series of experiments on tadpoles in which the posterior limb rudiments had just appeared. He found that when the anlage of one side only was cut, three limbs were sometimes formed on that side instead of one. If the anlage of both sides were cut at the same time, six limbs sometimes resulted, an extra girdle with two legs being on each side. In certain regeneration experiments where a single cut was made, only one extra limb was formed, probably due to crowding, and this limb was always a mirror image of the nearer stem limb. When two extra limbs were formed, each was in symmetry with the other if the two were near together, but in symmetry with the nearest stem limb, if they were far apart. Tornier considered the number of parts formed, and the degree of union of the extra parts as dependent upon the space in which the parts developed. Thus, in a limited space, one part might be crowded out, or two parts become more or less fused.

In addition to his experimental work, Tornier carried on a comparative study of reduplication, particularly in insects and in mammals. He concluded that the results of his experiments on amphibia could be applied to the regenerated legs found in insects and to the supernumerary limbs of mammals. He showed that reduplications in insects could be explained as due to injuries resulting from wounds, and extra parts in mammals by a pressure of the amniotic fold upon the embryo. According to this view, the number of cut surfaces and the available space determine the number of supernumerary parts. Thus he explained the formation of one or two extra parts in both insects and mammals.

Further observations were made by Tornier concerning the symmetry of the extra parts in insects, and in mammals. In the beetle, he found that if two extra limbs develop, they are right

and left respectively, but that if only one develops, it is a mirror picture of the stem leg from which it arises. He furthermore states that the evidence from mammals also indicates that an extra limb is a mirror image of the stem limb.

The experiments and observations of Tornier seem to have been somewhat neglected. They are certainly highly instructive as indicating the fundamental similarity between the extra parts of insects and vertebrates. They are also interesting in that they afford comparison with the results obtained in the present work. Many of the reduplications found in *Drosophila*, had they been discovered as isolated cases, might readily have been explained as regenerations due to injuries, as Tornier has suggested for similar formations in beetles. It is rather striking that the symmetry of the regenerated parts of insects which Tornier has observed is like that of the extra appendages of *Drosophila*. There is, however, one main point of difference. In *Drosophila* the extra legs are not due to injuries, and they are fully formed when the flies hatch. The character is, moreover, inherited, in the sense that the potentiality for division of legs is present in every fly of the abnormal stock, whether or not it is actually realized. The natural conclusion, therefore, is that in the abnormal stock of *Drosophila* that A factor, acting with the rest of the organism, produces a condition favorable for bringing about repeated divisions of the leg anlage. The separation of the cells of the anlage, as the experimental work on *Drosophila* has shown, must be brought about more readily when a low temperature is maintained. The experiments have further shown that a low temperature is effective only if applied during the early larval stages. Since this is the case, the low temperature must act in some way not only on the cells of the anlage, but also on the cells which are to give rise to the leg anlage, for at the very early stages the imaginal discs have not yet been formed.

Other work on regeneration, besides that of Tornier, lends support to the conclusion that the formation of the extra parts may be due simply to a separation of the cells of the anlage. For example, Morgan found that, when the head of a Planarian

was cut off, a single head would regenerate, if no further cut were made. However, if a longitudinal slit were made at the anterior end, or if a wedge-shape piece were removed from the middle of the body, a new head would grow from each of the cut surfaces. The breaking of the solid connection between the cells thus determines the duplex nature of the regenerating end. It therefore seems probable that, in *Drosophila*, if the cells should be separated at the end of the developing limb, a complete bifurcation would result, and the structure would be duplex throughout a less or greater extent, depending on the stage of development of the anlage, or upon the length of the separation of the cells. If the separation should occur on the side of a developing limb, either one or two extra limbs might result, depending upon the degree of restriction of the parts.

INHERITANCE OF REDUPLICATIONS IN OTHER ANIMALS

1. Inheritance of extra toe in fowls

With the exception of fowls, little is known of the inheritance of reduplications, and the data collected do not conform to those of ordinary Mendelian inheritance. Davenport, Bateson and Saunders, Punnett and Hurst have done considerable work on the inheritance of extra toes in fowls. Davenport found that the extra toe character acts sometimes as a dominant, and sometimes as a recessive. His results were as follows:

Normal \times extra toe $\rightarrow F_1$ [21.6 per cent normal 78.4 per cent abnormal]
 F_1 normal $\times F_1$ normal $\rightarrow F_2$ [73.9 per cent normal 26.1 per cent abnormal]

Davenport concluded that the extra toe character is dominant to the normal, but sometimes so imperfectly so as not to appear. This imperfection of dominance, he found, occurs in homozygous as well as heterozygous individuals, for in some pure bred strains of Houdans or Dorkings having five toes on each foot 3 to 4 per cent of the offspring failed to develop the extra toe on either side. Davenport thinks this is due simply to a weakening of the dominance and not to its reversal. Bateson and Saunders, after working with the same character in the Dorking fowl, also concluded

that extra toe acts sometimes as a dominant, and sometimes as a recessive. They furthermore state that the recessive normal character may sometimes dominate. Bateson and Punnett found that a single individual may give different proportions of extra toed offspring at different periods.

Hurst's results on fowls are as follows:

In F_1 the extra toe of the Houdan is dominant over the normal foot of Leghorn, Hamburg and Cochin. In some cases the dominance is complete, the extra toe being reproduced in its entirety; in other cases the dominance is incomplete, all stages of extra toe being produced, from the most perfect toe down to the mere duplication of the nail; in a few cases the extra toe is found in one foot only, the other foot being apparently normal; in a few cases there is simply an elongated hallux. In F_2 the hybrid dominant extra toes of F_1 mated together gave dominant extra toes and apparently recessive extra toes in proportion 3.8:1; mated with pure recessive (no extra toes) they gave dominant extra toes and apparently recessive no extra toes in varying proportions 1:1.3, 1:1.1, 1:1.4, 1:3.3.

Hurst concludes that the 1:3.3 rates must be due to some abnormal disturbance, since the other results are so nearly uniform. He therefore regards the case as a true instance of failure of dominance, but thinks it evident that segregation takes place along Mendelian lines. Hurst finds that cases of incomplete dominance in fowls appear to be about twice as numerous as cases of complete dominance.

2. *Inheritance of polydactyly in man*

Polydactyly in man is apparently a dominant, but as Davenport states, polydactyly is difficult to explain on Mendelian principles. Bateson finds that it may appear in the same strain or family under forms morphologically very dissimilar. The general facts of inheritance of extra digits in fowls and in man are thus very similar to those for *Drosophila*, for in all three the more divided state may sometimes act as a dominant and sometimes as a recessive. In *Drosophila*, however, the dominance is to some extent dependent upon the environmental conditions. It seems probable that a similar explanation might apply to these other cases also, and that, to obtain a large number of abnormal

individuals, certain external conditions must be maintained throughout development. Whether or not these and other cases of reversal of dominance or incomplete dominance are the result of environmental conditions, can of course be determined only by future experiments.

3. All reduplications not dominant characters

Bateson has stated that the less divided state is usually dominant to the more divided states. Clearly the extra legs of *Drosophila* as well as the extra digits of fowls and of man, are exceptions to this statement. The mule hoof of swine, reported by Spillman, and the webbed character of the digits of man, reported by Newsholme, do, however, conform to Bateson's rule, since both of these dominate the more divided or normal condition. Thus the less divided state in some animals is a dominant, in others a recessive. I see no reason to suppose that the more divided state should, in all animals, act in the same way—as a recessive or as a dominant. Bateson considers the added divisions to be due either to “the addition of some factor or power which enables the part to divide,” or to the “absence of something which in the normal body prevents the part from dividing.” In the case of *Drosophila*, it need only be stated that some germinal change has brought about a new condition of the cells of the leg anlage favorable to bifurcation, but further than this we cannot go.

SUMMARY

1. Selection aimed to produce races of *Drosophila* possessing high or low numbers of teeth in the sex-comb resulted in the isolation of races with high and low numbers of teeth; but in neither race was the number of teeth of the average individual higher or lower than the extreme variants in the wild stock.

2. During the course of the selection a mutation, involving reduplications in the legs, appeared. The origin of the mutation was probably not determined in any way by the selection, for repeated selection in a new line was not followed by a similar mutation.

3. The new character was found to be due to a sex-linked factor, the location of which in the sex-chromosome is close to that of the factor for vermilion eyes.

4. The extra legs sometimes acted as a dominant, and sometimes as a recessive character, and flies homozygous for the reduplicating factor were often perfectly normal.

5. The reduplications were found to be due to some extent to the temperature, as a low temperature, maintained throughout the larval life, was necessary for the production of a large proportion of abnormal flies. Only flies carrying the reduplicating factor, however, could be thus affected by a decrease in temperature.

6. The extra legs were of a variety of types, and the number of extra parts in a single leg varied from one to four. A definite relation in symmetry was found to exist between the normal and the supernumerary parts. It is apparent that the extra parts are formed by one or more bifurcations, and it is suggested that reduplications in other animals may be explained in a similar manner.

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EXPLANATION OF PLATES

All the drawings were made with camera and Zeiss binocular, paired oculars 3, paired objectives a_3 , the right objective and ocular alone being used. The drawings are reduced one-third the original size, and represent a magnification of 57.2 diameters. The number of teeth in the sex comb is accurately represented. The figures of each of the three pairs of legs are arranged in the plates according to the number of bifurcations of the leg, and begin with those in which the most distal parts only are affected. Unless otherwise indicated, all legs except those drawn are normal, and all legs figured are drawn from ventral view.

PLATE 1

EXPLANATION OF FIGURES

1 Right first leg of wild male, showing coxa, trochanter, femur, tibia and tarsus of 5 segments. The first tarsal segment bears the sex-comb, the teeth of which always point toward the fly when the leg is bent as figured. Tarsus ends in a small pad and 2 claws which curve towards fly when leg is flexed.

2 Right lateral view of left first leg of very abnormal male, developed at low temperature; femur and tibia reduced and twisted, tarsus with 2 sex-combs, 17 and 6 teeth respectively, and 3 pairs of claws on the last of the 4 segments, which probably represents two fused segments. Symmetry L; R, L. Other legs broken or abnormal.

3 Left first leg of female; first three segments of tarsus much thickened, and prolonged distally into three branches of two segments each; claws missing. See also figure 35 which is right third leg of same fly.

4 Left first leg of male. First segment of tarsus very wide (with 3 sex-combs) giving rise to 3 branches of 4 segments each. Symmetry L, R, L. Inner branch a left with 2 claws, 14 teeth; middle branch a right with 2 claws, 13 teeth; outer branch a left with 2 claws, 19 teeth.

5 Left first leg of female; proximal part of leg normal; tarsus very short; small branch without claws proceeds from second tarsal segment; probably 5 segments in tarsus. See also figure 20 which is right first leg of same female.

6 Right first leg of male from ventro-lateral view; first tarsal segment bifid; inner branch duplex, as shown by 2 combs of 9 and 10 teeth, and 2 pair of claws; outer claws and comb left, inner right. Outer branch (a right) with a single comb of 11 teeth and with 2 claws. Symmetry R; L, R. The right third leg of same fly had a small elevation on the trochanter.

7 Right first leg of female; lower part of tibia bifurcated, each branch ending in a complete tarsus; outer tarsus (a right) with 2 claws; inner tarsus (a left) with 3 claws.

8 Right first leg of male; first segment of tarsus bifurcated, forming two tarsi; outer branch (a right) with 9 teeth, 2 claws; inner branch (a left?) with groups of 3 and 6 teeth, and incomplete distal portion; 5th segment evidently broken off. See also figure 37, which is left third leg of same fly.

9 Left first leg of male; tibia bifurcated as in figure 7, ending in two tarsi; outer branch (a left) with 11 teeth and 2 claws; inner branch with claws missing and with double comb on single tarsus. Double comb consists of a right outer of 9 teeth and left inner of 9 teeth. Symmetry L, R; L.

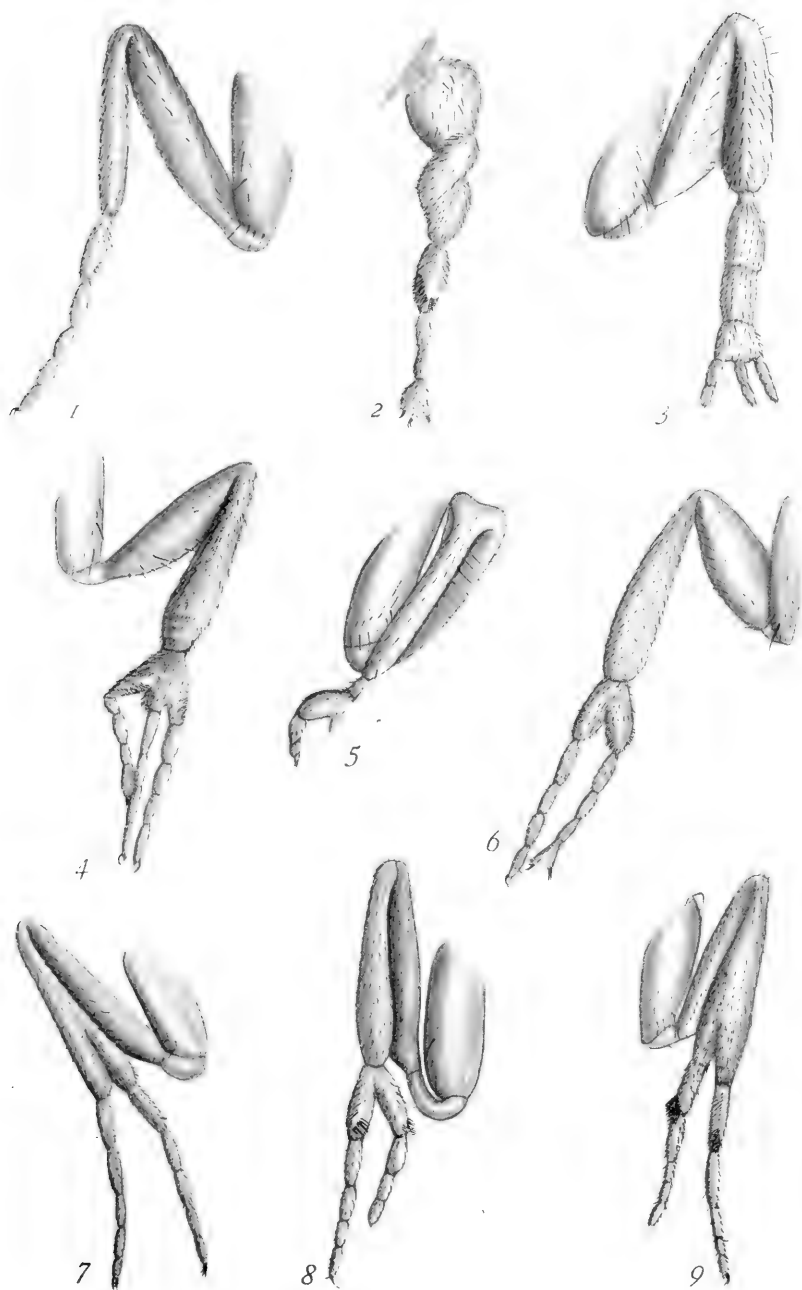


PLATE 2

EXPLANATION OF FIGURES

10 Right first leg of male; first tarsal segment with two combs, outer containing 16 teeth, inner 15. From this segment arise two branches, the outer again bifurcated at the second segment, the inner single. Symmetry of the three pairs of claws somewhat uncertain, but probably right, left and right respectively; outer comb probably equivalent to a right and a left combined. Symmetry R, L?; R.

11 Left first leg of male; tibia cleft at distal end, giving rise to a single outer branch (a left) with 12 teeth and 2 claws, and an inner branch, which bifurcates again into a right outer with 10 teeth and 2 claws and a left inner with 13 teeth and 2 claws. Symmetry L, R; L. Apparently this leg is incapable of bending between femur and tibia.

12 Left first leg of male; femur and tibia undivided as in figure 10, but rather broader than normal; tibia ending in two tarsi, the outer duplex, and apparently twisted through 180°, so that the comb is below; inner branch a left with 10 teeth; symmetry of outer duplex branch uncertain.

13 Left first leg of male; tibia split at distal extremity, giving rise to an inner single branch (a left) with 12 teeth and 2 claws and an outer branch with a large segment containing a comb of 21 teeth, which is probably equivalent to a right and a left, and ending in two branches of four segments each. Outer tertiary branch a left with 2 claws, inner a right, also with 2 claws. Symmetry L; R, L.

14 Left first leg of female from lateral ventral view; coxa reduced, trochanter not visible; femur duplex, of very abnormal shape, and ending in one normal tibia and tarsus (left) and one abnormal tibia, apparently fused with the femur, bent upon itself, and ending in a right tarsus. Symmetry L, R(?).

15 First pair of legs of male; tibia of right leg split as in figure 11, to which it is similar; outer branch from tibia (a right) with 13 teeth and 2 claws; inner branch with two combs of 17 and 14 teeth respectively; inner branch bifurcates at second tarsal segment and ends in two tertiary branches with two claws each. Symmetry R; L, R. Femur of left leg gives rise to a left normal leg with 12 teeth and 2 claws, but has also a small, incomplete and duplex branch. Branch has two groups of 10 and 11 teeth on its first segment; tibia of branch may be fused with this segment, or may be represented by the elevation on the femur from which the branch arises. Symmetry L, R; L. See also figure 34, which is right third leg of same fly.

16 Right first leg of male; coxa overlapped by thoracic plates, usually not represented; femur has a branch from its proximal end, but otherwise gives rise to a normal right leg, with 11 teeth and 2 claws. Branch from femur ends in a short tibia and tarsus, the last segment of which is bifurcated; tarsus of branch has a double comb of 6 and 9 teeth and each of end segments has a pair of claws. Double comb and claws are lefts and rights. Symmetry R; L, R. Left third of same fly missing.

17 Right first leg of male; femur, ending in a normal tibia and also with a branch from the posterior surface; normal tibia ends in two tarsi; an outer left with 8 teeth and claws missing, and an inner right with 12 teeth and 2 claws; branch from femur undivided, but with a double sex-comb of 10 and 9 teeth, which are left and right respectively. Symmetry L, R; L, R.



PLATE 3

EXPLANATION OF FIGURES

18 Right first leg of male; trochanter indefinite, femur ending in a normal right tibia and tarsus, but with a large duplex branch from its proximal end; normal tarsus with 12 teeth and 2 claws; outer branch a left with 7 teeth and 2 claws, and with tibia probably fused with femur; inner branch a right with 10 teeth and 2 claws. The main axis of the inner branch is in a more ventral plane than that of the outer. Symmetry R; L, R.

19 First pair of legs of female, completely fused with each other; two separate coxae, trochanters scarcely visible; femora of two sides completely fused with the exception of outer femur of right leg, which is capable of separate motion. *Right* leg with duplex femur, and 2 tibiae and tarsi; outer femur, tibia and tarsus are those of normal right leg with two claws; inner femur of abnormal size, tibia bent upon itself, and produced into a normal left tarsus with two claws. *Left* leg with two completely fused femora of abnormal size; *inner* femur ends in a single tibia with two tarsi, the inner of which is a left with two claws, the outer incomplete; *outer* femur ends in three tibia which are left, right (?) and left respectively; each of these tarsi has two claws. Symmetry of double leg R, L : R, X; L, R?, L.

20 Right first leg of female; single coxa, trochanter not visible; two femora, absolutely fused, and prolonged in opposite directions. *Outer* femur ends in a normal right tibia and tarsus, with two claws; *inner* femur ends in a bent left tibia and a thickened tarsus with two claws. Symmetry, R, L. See also figure 5, which is left first leg of same fly.

21 Left first leg of male; trochanter small, femur bifurcated at its proximal end. Outer part of femur ends in a normal left tibia and tarsus with 11 teeth and 2 claws; inner part of femur is produced into 2 small tibiae, an inner undivided right, the tarsus of which has 29 teeth and 2 claws, and an outer, which ends in a proximal tarsal segment with 32 (?) teeth. From this segment arise two branches, the outer incomplete and again branched, the inner with 4 segments and 1 claw. Symmetry R : X; Y, Z, L.

22 Left first leg of male; coxa and trochanter single, two distinct femora from trochanter; outer a left with normal tibia and tarsus, latter having 11 teeth and 2 claws; inner a right, short and broad, with small tibia, and incomplete tarsus having 10 teeth. Symmetry R, L.

23 Right first leg of male; trochanter enlarged, and giving rise to two femora; outer femur normal and produced into normal tibia and tarsus with 11 teeth and 2 claws; inner femur and tibia short and broad and not bent upon each other; proximal segment of tarsus of inner part bearing two groups of 15 and 9 teeth respectively. This segment ends in 2 small branches, apparently of 2 segments and 1 claw each; outer claw and comb are rights, inner lefts. Symmetry R; L, R.

24 Left first leg of male; coxa double. This specimen had thus two left first legs almost separate from their bases; outer leg with no visible trochanter, and having an enlarged femur ending in two tibia; outer tibia of outer leg normal, ending in normal left tarsus with 12 teeth and 2 claws; inner tibia of outer leg bent upon itself and ending in nearly normal right tarsus with 11 teeth and 2 claws; inner leg with distinct trochanter, left femur and tibia, and incomplete tarsus. Symmetry L; R, L.

25 First pair of legs completely fused, from very abnormal fly, raised at low temperature. Coxae and trochanters may be represented by part above the fused segment, which stands for femora and tibiae. Two normal tarsi, a right with 11 teeth and 2 claws, and a left with 11 teeth and 2 claws, proceed from the long fused segment. All other legs of this fly were either abnormal or broken off.

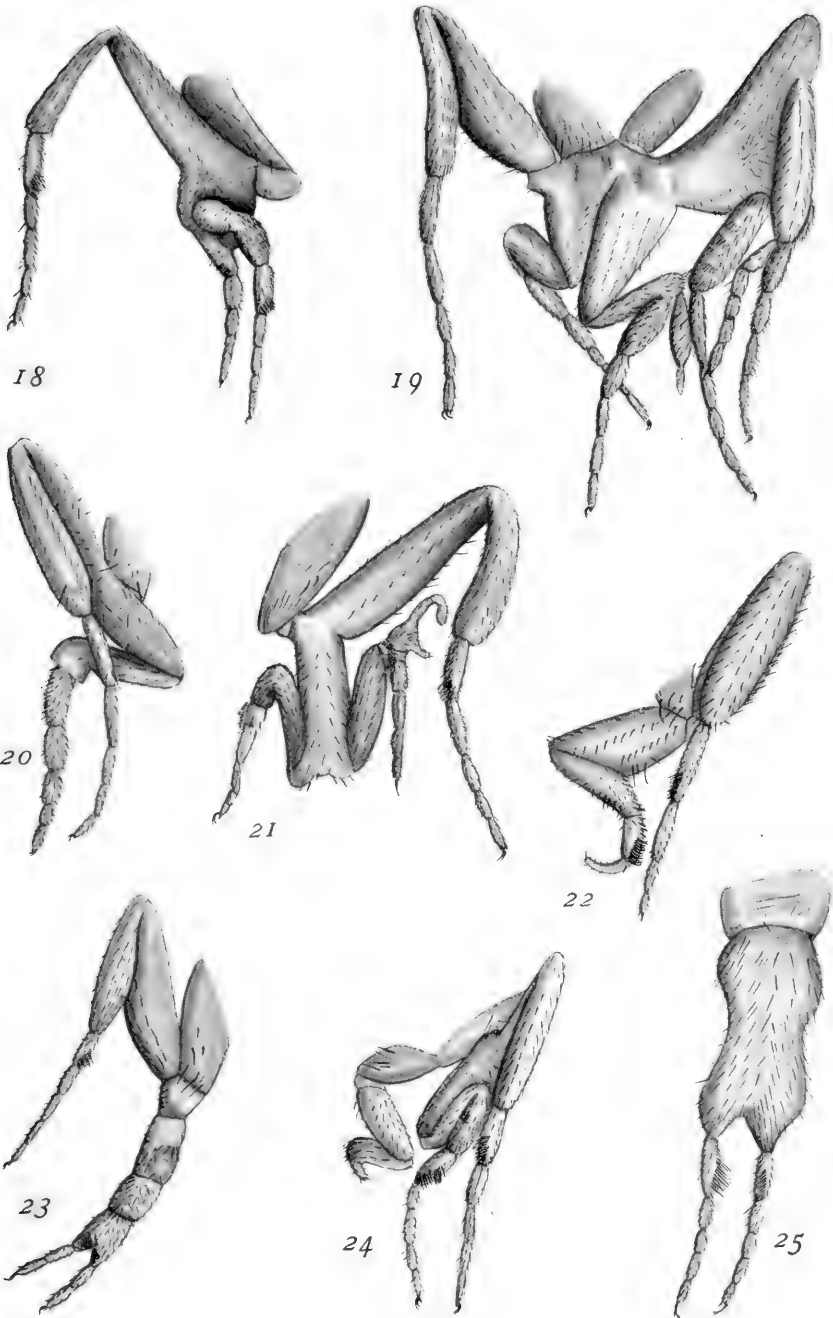


PLATE 4

EXPLANATION OF FIGURES

26 Right first leg of male fly raised at low temperature; coxa and trochanter indistinct, femur short and thick, tibia bent; tarsus without extra segments but with three discontinuous combs linearly arranged and having 4, 5 and 6 teeth respectively. Right first of same fly bifurcated.

27 Left first leg of male fly raised at low temperature; coxa and trochanter indistinct; femur and tibia short and thick; no extra parts. Other legs of same fly abnormal.

28 Left first leg of male fly raised at low temperature; coxa and trochanter indefinite, femur short and round, tibia short, lower part of leg probably broken off. Other legs of same fly were broken.

29 Right second leg of wild male, showing same parts present in first leg of wild fly, but without sex-comb in the tarsus (compare figure 1). The second leg of a normal male is identical to that of a normal female.

30 Left second leg of male; coxa distinct, trochanter partly divided, each of two parts giving rise to a femur; outer femur normal, ending in normal left leg with 2 claws; inner femur shorter, ending in a bent right tibia with thickened tarsus having 2 claws. Symmetry R, L.

31 Left second leg of male; tibia thick and bifurcated at its distal end, from which proceed 2 tarsi; outer tarsus a normal left with 2 claws; inner tarsus with single proximal segment which bifurcates into two branches of 4 segments each, the outer a right with 2 claws, the inner a left with 2 claws. Symmetry L, R; L.

32 Left third leg of male, with all parts abnormally formed, and relation of proximal segments indefinite; left first of same fly broken.

33 Right third leg of wild male, also without sex-comb.

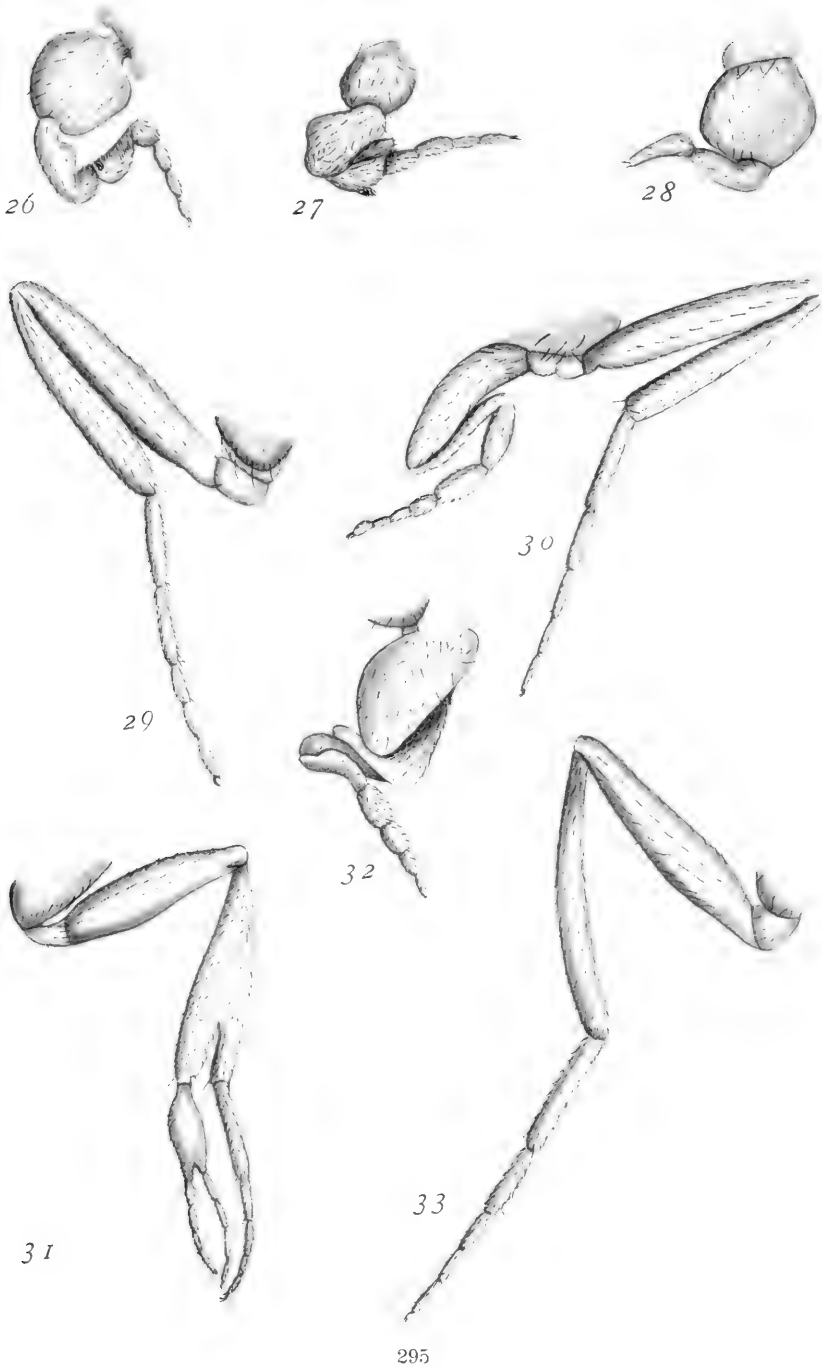


PLATE 5

EXPLANATION OF FIGURES

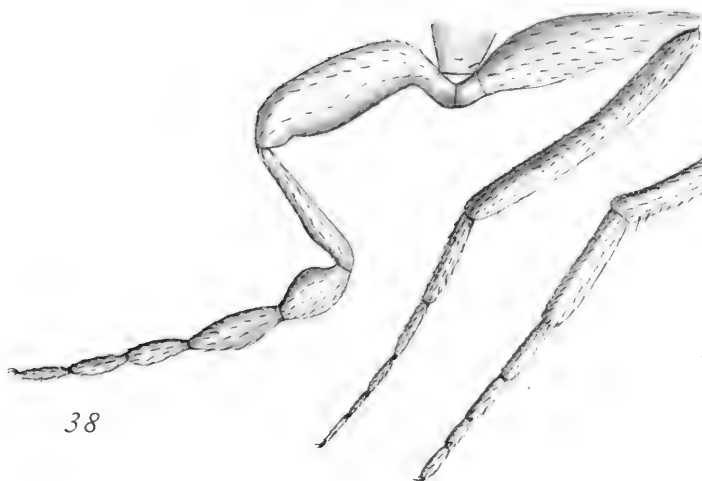
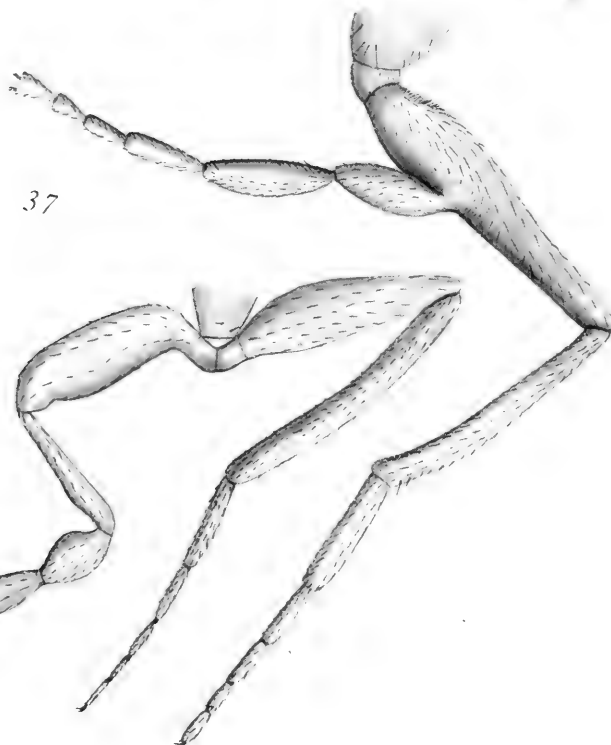
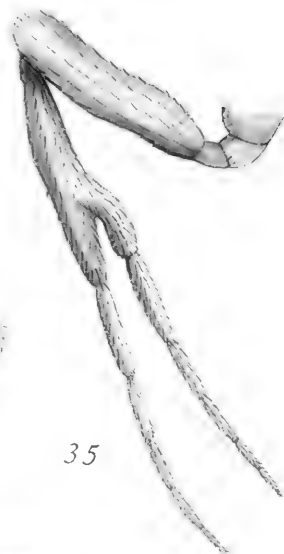
34 Left third leg of male; first tarsal segment enlarged and giving rise to two branches, outer with 4 segments and inner incomplete, and claws of both missing. See also figure 15, which is first pair of legs of same male.

35 Right third leg of female; lower part of tibia bifurcated, giving rise to two complete tarsi with indefinite symmetry. Inner tarsus with two claws. See also figure 3, which is left first of same female.

36 Stump of right third broken leg of male raised at reduced temperature; figure shows abnormal shape and size of basal parts; distal parts broken off. See also figure 27, which is left first of same fly.

37 Left third leg of male; femur ending in normal left leg with two claws, and also having a large branch from its posterior surface; tibia of branch short and giving rise to tarsus, which has two pair of claws, a left and a right respectively, on the most distal of its five segments. Symmetry L, R; L. See also figure 8, which is right first of same fly.

38 Left third leg of female; coxa and trochanter single, latter giving rise to two femora; outer femur a left, produced into normal tibia and tarsus with 2 claws; inner femur a right, produced into a short tibia which ends in a thickened tarsus with 2 claws. Symmetry R, L.



AN EXPERIMENTAL STUDY IN CLEAVAGE

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FIFTY FIGURES (THREE PLATES)

If the eggs of sea-urchins are shaken violently a few minutes after fertilization (from 4 to 7 minutes is the most favorable time) in a varying proportion of cases the centrosome will fail to divide and eggs with only one division center are obtained. A brief description of these monaster eggs was given by Boveri ('03) and later by M. Boveri ('03) but up to the present time no work has appeared which dealt in detail with this interesting phenomenon. At the suggestion of Professor Boveri, I took up an experimental and cytological study of monaster eggs in order to gain more light on the mechanics of cell division.

The cytological part of the work involves so many special questions that it seems wiser to reserve it for a separate paper. The present study deals with the cleavage of the monaster eggs and with some experiments which were made in order to gain light on the processes concerned. On the basis of these experiments the conclusion seems warranted that *at the time of fertilization progressive changes, which go on independently of the nucleus and of cleavage, are initiated in the cytoplasm of the eggs and that these changes determine the position of the spindles in the egg and consequently in its blastomeres.*

The experimental work was done in the Zoölogical Station at Naples, during the spring of 1914, while I was occupying the Smithsonian table there. To both of these institutions my thanks are due. Especially to Professor Boveri, however, I

wish to express my appreciation both for suggesting the problem and for advice given during the course of its completion.¹

For the sake of clearness, it will be necessary to give a brief account of the changes going on in the monaster eggs prior to their cleavage. After the eggs have been fertilized and shaken, they are put into a glass dish with normal sea water and left undisturbed until signs of division appear. When the two asters show in a majority of the eggs, in a perfectly normal manner, a certain proportion of the eggs will be found with only one sphere. This is very large (much larger, in fact, than the single aster of the normal eggs) and lies in the center of the egg. Sections show that the chromosomes are attached to this single sphere on one side, by means of the fibers which radiate out from it (fig. 1). The condition, shown in the figure, persists for a variable length of time, but usually when the first division begins in the normal eggs the chromosomes split and slowly separate. Following this the aster becomes flattened and concave on the side next to the chromosomes and it moves towards the surface of the egg, the convex side forward. This retreat of the aster is slow and as it takes place the chromosomes form their vesicles and fuse to form one nucleus with, of course, the double number of chromosomes. While these processes are taking place within the egg, on the side opposite that towards which the aster is retreating, the protoplasm begins to undergo a series of movements when blunt pseudopod-like processes are thrown out (fig. 2). The intensity of the movement is variable in different eggs, but occasionally it may lead to the complete cutting off of protoplasmic balls, and these may remain separated from the egg permanently (fig. 16). The length of time this movement continues also is variable, but as the astral radiations begin to disappear, the pseudopod-like processes are withdrawn and the egg assumes its normal rounded form.

Not all monaster eggs pass through the changes described above. In any given experiment, one usually finds a small

¹ Although the present work was done while I was working with Professor Boveri, the paper was not written until my return to America. It has been impossible to submit this manuscript for his approval, before it goes to press.

number of eggs in which the monaster condition shifts very quickly to the amphiaster and the egg divides normally though a trifle behind the controls. The cytological details of this have been followed and will be given in a later paper.

The monaster eggs, after they have assumed their normal rounded form, following the protoplasmic movements, show a marked difference in their behavior and we may distinguish three general classes: (a) In part of the eggs, at the next division cycle, a monaster reappears and the egg goes through the same process as before, except that the protoplasmic movement at this time is usually much more severe; (b) In other eggs the monaster condition goes over into a triaster or tetraster condition with the subsequent abnormal development characteristic of these eggs; (c) Finally, and this class includes the majority of the eggs, at the next division cycle, an amphiaster is formed and the eggs divide giving rise, in many cases, to perfectly normal plutei. It is the cleavage of this last type of monaster egg which we propose to take up in the present study.

Boveri, in his first paper on this subject, describes very briefly the cleavage of these monaster eggs. He found a wide departure from the normal type, the clearest cases being those in which the eggs cleaved like $\frac{1}{4}$ -blastomeres, that is, with the production of the micromere in the 4-cell stage.

Boveri's shaking experiments were repeated a number of times and large numbers of monaster eggs were isolated and followed through their cleavage. There was a great variation in the time at which the monaster eggs recovered and went over into the amphiaster, as well as in the cleavage itself. As shown below, however, I found that there was a correlation between the time when the monaster eggs divided, as compared with the control eggs, and the type of cleavage which they showed. That is, eggs dividing at the same time, with reference to the controls, showed the same type of cleavage although the experiment was made on different females and at different times. The variation in the times when the monaster eggs would divide ranged from the telophase of the first division to the 16-cell stage of the controls.

CLEAVAGE OF MONASTER EGGS

Taking up first the class of monaster eggs which shifted very quickly over into the amphiaster condition, I found that the cleavage was perfectly normal in every detail and such eggs could not be distinguished from the controls except when they were isolated at an early stage. It is worth while to mention here also that these eggs did not pass through a period of protoplasmic movement and that the normal number of chromosomes was present in the blastomeres of the 2-cell stage.

The division of the monaster eggs which had passed through a period of protoplasmic movement, on the other hand, was never normal.

The first division took place very slowly and there was always a more or less intense protoplasmic movement in the division plane with the temporary formation of finger-like processes, as shown in figure 3. Occasionally, and this seems to be associated with an excentric position of the spindle, large masses of protoplasm would be cut off from the egg and when the separation of the two blastomeres finally took place, this protoplasmic ball remained permanently detached (fig. 4). This demonstrates the necessity of following the eggs individually through cleavage, for in later stages this enucleated mass was not to be distinguished from a small blastomere unless the previous history of the egg was known. The result of the first division was two blastomeres of unequal size (fig. 5). The controls were usually in the late 4-cell stage at this time.

The second division of the monaster eggs was again accompanied by protoplasmic streamings in the division plane of the blastomeres, but ultimate separation took place and after rounding up four cells, two of which were larger than the others, were found (fig. 6). The controls were either in the late 8-cell or the early 16-cell stage at this time.

The third division was variable; the spindles of the blastomeres showed no constant relation to one another, as they do in the normal eggs, and the result was an irregular mass of cells, as shown in figures 7 to 13. We find the blastomeres of this 8-cell stage in one of three conditions. Either there are two very small

cells which have been given off by the larger blastomeres of the 4-cell stage (figs. 7-9) or we find only one of these small cells (figs. 10-11), or the four blastomeres have divided more or less equally with the formation of four large and four small cells (figs. 12-13). In figure 12, this size relation is not so clear. By the time these monaster eggs had reached the 8-cell stage the controls were in the 16- or 32-cell stages.

The 16-cell stage of the monaster eggs was invariably a simple splitting of the blastomeres of the 8-cell stage. When two small cells were present in the earlier stage (as will be shown later these cells are to be considered as micromeres) we find four of them in the 16-cell stage. If only one small cell was present in the 8-cell stage, then two were to be found in the next division, and no new micromeres were given off. In the event that there were no micromeres in the 8-cell stage, the later division was an equational one, in all the cases that I followed. However, my observations on such eggs are not sufficiently extended to allow me to state that the micromere formation is invariably suppressed.

Boveri describes cases where he obtained the production of a micromere in the 4-cell stage. This is figured in a later paper (Boveri '10). I did not find any such eggs in the course of my experiments at Naples during the past spring, although, as will be shown in the latter part of this paper, such eggs may be produced at will by the use of suitable narcotics.

In the same female one usually finds the same type of cleavage as regards the early production of one or two micromeres. In the majority of these experiments carried on at Naples, only one micromere was formed in the 8-cell stage.

From monaster eggs perfectly normal plutei may be obtained, although comparatively few embryos reach this late stage of development.

After it was found that the micromeres could be produced early, it became a point of great interest to know how the cleavage planes of the blastomeres behaved with regard to the original polarity of the egg. Boveri ('01) has shown that the pigment ring, in the eggs of *Strongylocentrotus lividus*, marks out the original axis of the egg. In his paper dealing with the monaster

eggs, already referred to, he states that in all of the cases followed by him, the aster retreated in the original axis of the egg.

In order to settle this point, a female was selected whose eggs showed the ring very clearly, and after shaking, a number of monaster eggs were followed individually. I could not convince myself, however, that there was any constancy in the behavior of the eggs in this regard. I found cases where the aster had retreated in the plane of the egg, as shown in figure 14, but just as many cases were noted where it had moved in a plane at right angles to this (fig. 15) or in a plane forming an acute angle with the original axis of the egg, as this is marked out by the pigment ring. It was necessary to follow the course of the aster at this early time, for after the protoplasmic movement has set in, the pigment ring is scattered and it is not possible to tell later, just where it lay. In the cases followed the amphiaser took up a position at right angles to the plane in which the monaster had retreated and this would indicate that the division of the egg was independent of the polarity which the egg undoubtedly possesses.

The cleavage of the monaster egg is most strikingly different from that of the normal in the production of the micromere in the 4- or 8-cell stage. In order to explain these results, Boveri ('05) advanced the suggestion, that at the time of fertilization changes were initiated in the cytoplasm which went forward independently of the nucleus and that these changes controlled the position of the spindles in cleavage. He says:

Diese Tatsachen lehren (he is speaking of the early formation of the micromeres in the monaster eggs) wie nebenbei bemerkt sein mag, dass die typische Aufeinanderfolge von Spindelstellungen und damit von Teilungsebenen nicht in einer dauernden festen Eistruktur begründet ist, sondern dass die Konstitution des Eies während der Entwicklung und infolge Einleitung der Entwicklungsprozesse bestimmt gerichtete Veränderungen erfährt, welche der Reihe nach verschiedene gegenseitige Lagerung der Teilungszentrum bewirken. Es gibt bei dieser Umwandlung der Eistruktur eine Periode, während deren die Spindeln in der äquatorial Ebene (karyokinetischen Ebene) des Eies liegen, dann eine solche, wo sie zu dieser senkrecht stehen u. s. w. Wird, wie es im Monasteri der Fall ist, die Entwicklung, d. h. der Ablauf der karyokinetischen Vorgänge eingeleitet, ohne dass es zunächst zu einer Vermehrung des einfachen Zentrums und damit zur Kern- und Zellteilung kommt, so wird die erste Periode der horizontalen

Spindelstellungen zum Teil oder ganz übersprungen und das Ei ist, wenn es nun die Teilung beginnt, so verändert, dass es der normalen $\frac{1}{2}$ -oder $\frac{1}{4}$ -Blastomere entspricht und sich wie diese furcht. Zellen-Studien V., p. 17.

The cleavage of the monaster eggs followed in the present study was in entire accord with the explanation advanced by Boveri, but a second possibility was not excluded. In the eggs which passed through the monaster cycle before they divided, there had been a doubling of the number of chromosomes so that in the 2-cell stage we had the chromosome complex of the 4-cell stage, and similarly, in the 8-cell stage the chromosome complex of the 16-cell stage. It seemed just as possible that the presence of the chromosome complex of the later stages might have some effect in the production of the micromere.

In order to clear up the question, a series of experiments were begun, the object of which was to retard or inhibit nuclear activity after fertilization for a varying length of time in order to see if the micromeres were produced early. For this purpose three methods were employed, cold, lack of oxygen, and certain narcotics. The method of experimentation was the same in every case. After fertilization the eggs of *Strongylocentrotus* were divided into two lots. One portion was allowed to develop normally, while the other was subjected to experiment. Whenever it seemed advisable, individual eggs were isolated and followed.

EXPERIMENTS WITH COLD

After membrane formation, a portion of the fertilized eggs was placed in a dish surrounded by ice and allowed to remain there while the controls passed through the first and second divisions. The temperature at which the eggs were kept was about 2°C. When the controls were in the 4-cell stage the treated eggs were brought to the room temperature and allowed to divide. The cleavage of these eggs was perfectly normal and the micromeres were not formed until the 16-cell stage, although, by this time the controls were young blastulae. The experiment was repeated several times on the eggs of different females with the same result in every case.

EXPERIMENTS WITH LACK OF OXYGEN

Loeb, in a large number of works, has shown that the addition of a small amount of potassium cyanide to sea water totally inhibits oxidation in the eggs of sea-urchins without causing any injury, provided the eggs are not exposed too long to its action. Since this afforded the simplest method of depriving the eggs of oxygen, it was employed in the following experiments. A portion of eggs were placed in a 0.0025 per cent KCN solution in sea water. When the control eggs were in the 4-cell stage, the treated eggs were removed from the solution, washed free from the KCN in fresh sea water and allowed to develop. In such cases the cleavage was perfectly normal and the micromeres did not appear until the 16-cell stage. This experiment was repeated a number of times.

EXPERIMENTS WITH NARCOTICS

The first narcotic used was chloral hydrate. The eggs were placed in a very weak solution of this drug, shortly after fertilization and were not removed until the controls were in the 4-cell stage. When released, the treated eggs gave a few cases where the micromeres appeared early, that is, in the 8-cell stage. This narcotic was quickly discarded for phenyl urethane, which, as Warburg ('11 a, '11 b) has shown by a careful series of measurements, does not materially retard the cytoplasmic oxidation, while it almost inhibits nuclear-division. Loeb and Wasteney ('13) have shown the same to be true for chloral hydrate and a number of other narcotics.

Various strengths of the phenyl urethane solution were used ranging from 1/2,000N. to 1/500N. After a few experiments, the weaker solution was used altogether because the cytoplasmic oxidation was less affected by this, and furthermore, in many females, the stronger solutions tended to produce cytasters in the eggs when they were transferred to normal sea water. In the experiments here recorded, the eggs were placed in the phenyl urethane solution thirty minutes after fertilization. At this time the spindle was usually formed.

The weak solution of the narcotic did not totally inhibit nuclear division. Some eggs were more affected than others so that when they were finally washed free from the narcotic (the controls were in the 4-cell stage), part of the eggs showed two nuclei (fig. 17) while in others the first division mechanism (aster, etc.) was still present. From the former eggs 'pseudotetrasters'² were formed and a division into 4-cells took place (fig. 19). In the latter case, the eggs divided into 2 cells. Not all the eggs treated with phenyl urethane reacted in the same way, there was always a varying proportion which formed tetrasters, triasters, or monasters but as a rule these abnormal eggs were in the minority. Different females showed a marked variation in this regard.

The results of March 7 may be taken as typical for the experiments with phenyl urethane.

On March 7, half-an-hour after fertilization, the eggs of a female were divided into two portions, one of which was placed in a 1/2,000N. solution of phenyl urethane, in sea water (this is about a 0.008 per cent solution). When the control eggs were dividing into 4 cells, the treated eggs were washed free from the narcotic by changing the water six times. Of course the eggs were allowed to remain in each change of water until they had settled.

In the treated eggs about an equal number divided into 2 and 4 cells, in the latter case through the formation of pseudotetrasters.³ Perhaps 10 per cent of the eggs did not develop at all or formed monasters, triasters, etc.

A large number of both the 2- and 4-cell stages were isolated and followed individually.

² The term 'pseudotetraster' is the same as used by Wilson ('01) for two spindles in the same egg which are independent of each other. In such cases the two amphiasters are not connected by mantle fibers.

³ While working in Naples, I was led to believe that real tetrasters were not formed as a rule in the eggs of *Strongylocentrotus*, after treatment with phenyl urethane. Since returning to America, I have repeated these experiments on the eggs of *Arbacia*, and sections from this species show that real tetrasters are much commoner than was thought. I cannot state what proportion of the eggs of *Strongylocentrotus* formed real tetrasters.

In figures 20 to 28 the cleavage of three typical eggs is given, eggs which had divided into 2 cells after treatment with phenyl urethane. At the time of the first division, it should be emphasized, that the control eggs were in the 8-cell stage. The treated eggs divided into two blastomeres of unequal size, as may be seen from a comparison of figures 20, 23 and 26. This inequality in the relative size of blastomeres was very constant, although the proportions varied.

When the controls were in the 16-cell stage, with a few eggs in the 32-cell stage, the treated eggs divided again to form a 4-cell stage (figs. 21, 24, 27). An inspection of these figures will show that one very small cell was given off from the larger blastomere; this is most striking in the cases shown in figures 24 and 27.

In the next division of the eggs, there was a simple splitting of the pre-existing blastomeres, so that in the 8-cell stage there were two micromeres, four mesomeres and two macromeres present, as shown in figures 22, 25 and 28.

In the 16-cell stage of the treated eggs, there was again a simple splitting of the blastomeres of the 8-cell stage, no new micromeres being formed.

In the experiments of March 7 a large number of the eggs in the 2-cell stage were isolated after treatment with the narcotic and only rarely did the micromere fail to appear in the 4-cell stage, as described above. In these rare cases, one or two small cells would appear in the 8-cell stage, as is shown in figures 29 to 31.

In figures 32 to 34 is given a very interesting case where two micromeres were produced at the 4-cell stage. I have found only one case of this sort.

The cleavage of the treated eggs which divided directly into 4 cells is given in figures 35 to 41. As in the case of the eggs of the 2-cell class, a large number of these were isolated and followed individually.

After division has taken place and the blastomeres have rounded up, one usually finds that two of the blastomeres are larger than their mates (figs. 35; 37, 39). This is nearly always the case, although here and there eggs appear in which one blastomere is small, two are medium-sized and one is large. The

cleavage of both types of eggs is the same. As was the case with the first type of treated egg, the controls were in the 8-cell stage at the time the pseudotetrasters divided.

When the controls were in the 16- to 32-cell stages, the treated eggs divided again, forming, of course, 8 cells (figs. 36, 38, 40).

In the 16-cell stage, there were two micromeres, eight mesomeres and six macromeres present, showing that a simple splitting of the blastomeres of the 8-cell stage had taken place. There was always some difficulty in distinguishing what cells were macromeres and what mesomeres, as the sizes of these two types of cells varied, but the micromeres were easy to see.

In figures 39 to 41 is given the cleavage of a pseudotetraster egg, treated in the way described with phenyl urethane. It may be taken as typical of the experiments carried on March 9. I may add that these experiments with narcotics were repeated a great number of times while I was in Naples and I never failed to get the early production of the micromeres, in at least a small percentage of the eggs. In the most favorable case, I got 96 per cent of the treated eggs in the 8-cell stage showing one or two micromeres.⁴

In all of the experiments eggs were obtained which did not divide until very late, after they had been washed free from the narcotic. The first division of such eggs is shown in figures 42 and 43. The later cleavage of these eggs was totally abnormal, but since the small cell appeared after the controls were in the 16-cell stage, think I we are reasonably safe in regarding it as a micromere.

Eggs treated with phenyl urethane may give rise to free swimming gastrulae but I did not attempt to rear them farther than this.

An attempt was made to determine the position which the spindles of the treated eggs took when they divided. Here again the same difficulty was encountered that was found in the monaster eggs. During the cleavage process the pigment band was

⁴ I have repeated these experiments at Woods Hole on the eggs of *Arbacia* and have obtained the early production of the micromeres. However, this material is not as favorable for study as the species used in Naples.

almost invariably so scattered that after the blastomeres had rounded up, one could not determine with certainty just where it lay. A study of a limited number of cases, where the scattering of the pigment was not so pronounced, is given below.

In the eggs which divided into 2 cells after treatment with the narcotic, the majority divided approximately meridionally, as shown in figure 44, but quite a number of cases were noted where the axis was nearly horizontal, or lay in a plane between the two (fig. 45). The position of the spindle did not seem to affect the production of the micromere, as the latter always came from the larger blastomere.

In the pseudotetraster eggs I was unable to establish any definite relation between the cleavage planes and the original axis of the egg, as this is shown by the pigment ring. Even when the eggs were first removed from the narcotic, the position of the nuclei or of the spheres was variable; quite frequently a line connecting the two would stand nearly at right angles to the pigment ring (fig. 46). After division the four blastomeres lay at any plane with relation to the pigment ring, as may be seen by comparing figures 47 to 50. In figure 47 two larger and one smaller cells contain this band; in figure 48 only the two large blastomeres have it; in figure 49 the two smaller and one larger blastomere possess it, while in figure 50 all the cells show pigment.

By the time the micromeres are formed, the pigment band is so scattered that it is impossible to determine from what region of the egg they came. However, no matter how much pigment the cell from which they came possessed, the micromeres never contained any trace of the pigment band.

DISCUSSION

A closer examination of the observations recorded above brings out several points of interest. In the normal sea-urchin's egg the micromeres do not appear until the 16-cell stage, but, as we have seen, these small cells may be thrown in the 4- or 8-cell stages if the egg has passed through a monaster cycle or has been treated with certain narcotics, such as chloral hydrate or phenyl urethane. The question arises: Is the formation of the

micromere due to the same cause in every case, and, if so, what is this common cause?

Obviously, the cleavage process itself has nothing to do with the micromere formation, since the latter may appear in the 2-, 4-, or 8-cell stage.

It is also quite clear that the nucleus with its chromosomes can play no direct part here. In the case of the monaster this factor could not be eliminated entirely. But in the eggs treated with phenyl urethane the case was quite otherwise. This narcotic inhibited nuclear division and we obtained eggs in which the micromere appeared in the 2-, 4- or 8-cell stages. In all these cases, the nuclear division had simply been held back, and when the micromeres were in the 2- or 4-cell stage, the normal chromatin complex of these two stages was present.

With the elimination of the cleavage process, and of any part which the chromosomes might play in the production of the micromeres, we are brought to the conclusion that the factors which control the production of these small cells lie in the cytoplasm, and the experiments recorded show that the process depends upon cytoplasmic oxidation.

When eggs were fertilized and subjected to a low temperature for a short length of time, it was to be expected that on the restoration of normal conditions they would divide normally, because it is well known that a lowering of temperature retards the rate of oxidation in animal cells. I have found no record of measurements of the oxidation rate in *Strongylocentrotus* eggs at 2°C., but Loeb and Wasteneys ('11) showed that it took the eggs of *Arbacia* 498 minutes to cleave when the temperature was 7°C., and it seems probable that at 2°C. the oxidation rate would be practically nil. Hence, in our eggs, oxidations and other activities were very much reduced, the eggs were fixed, as it were, in their development and the latter did not make any appreciable progress until the temperature was raised; then the cleavage was normal.

Potassium cyanide, while acting in an entirely different way, accomplished the same end result. This chemical reduced the rate of oxidation to zero by removing all free oxygen from the

solution in which the eggs were placed. Again the eggs were fixed in their development until normal conditions were restored. Then the cleavage was normal, the eggs taking up their development where it had been left off.

In both of these cases the lowering of the oxidation rate resulted in a stopping of the development of the eggs.

With the phenyl urethane, on the other hand, we have a substance which allows us to differentiate between two processes going on in the egg. This narcotic allows the cytoplasmic oxidation to go on at a practically undiminished rate, while it greatly retards nuclear activity. Its action amounts to a holding still of the nucleus while the cytoplasm goes through its changes (oxidations). After the narcotic is washed out of the eggs, they divide; their cleavage is not normal, but is like that through which the control eggs are passing at the time. And the micromeres are formed at approximately the same time, it makes no difference, so far as I have been able to determine, whether the treated eggs are in the 2-, 4- or 8-cell stage.

Thus the conditions for the early production of the micromeres seem to be two. First, oxidation must go on in the cytoplasm, and, second, a certain time must elapse after fertilization, a period extending from the time of fertilization to the 16-cell stage, as measured by the control eggs; these two conditions fulfilled, the micromeres appear.

In the monaster eggs these two conditions are fulfilled, the nucleus is held up in its division for a time while the cytoplasm goes through its changes. When the amphiaster finally appears, the division is controlled not by mechanical causes, such as we might imagine to be the case in the normal egg; but by the state of oxidation of the cytoplasm.

It is a very remarkable fact that when we place the spindle of the first division in an egg which has gone through cytoplasmic oxidation that is, in the 16-cell phase, so to speak—it takes up a position which will give the micromere. No mechanical explanation will be of service here, the only conclusion left being that there is present in the egg a structure, invisible but nevertheless real, which controls the position of the spindle. And that this

structure is in some way connected with the oxidation processes going on in the egg. Conklin ('12) working on the eggs of molluscs, has come to the conclusion that "The study of normal as well as of artificially altered cleavage points unmistakably to the conclusion that the position and axis of each spindle is fixed by the structure of the cell protoplasm, and since the position and axis of the spindle change regularly in successive divisions this protoplasmic structure must change regularly in the successive cell generations" (p. 8).

There are several interesting questions which present themselves with regard to the axes which the spindles of the monaster and phenyl urethane eggs took. Owing to the difficulties mentioned, it has been hard to get any satisfactory observations covering these points. The little evidence which I have, however, points to the general conclusion that the eggs tend to take up positions similar to those of the normal eggs when they are released, either from the narcotic or from the monaster condition.

Taking up the case of the eggs which divided into 2 cells after treatment with phenyl urethane, we must remember that when the eggs were placed in the solution the spindle was already formed. While in the narcotic, the nuclear division had advanced to some extent, more in some eggs than in others. When the narcotic was washed out, division followed more or less quickly. The control eggs were in the 8-cell stage at this time, that is, the cytoplasm was in the equatorial division phase. We should expect to find then the phenyl urethane eggs dividing in such a way that the cleavage plane would be horizontal. As a matter of fact, an examination of the eggs showed that the plane of cleavage might cut the pigment band at any angle (figs. 44-45). The division was, however, unequal and the micromere in the next division was formed from only one blastomere, as a rule. This shows that, as far as the micromere zone was concerned, the division was an equatorial one. Only in one case was the micromeral zone cut in two and here we got two micromeres in the 4-cell stage, as shown in figures 32 to 34. Such cases were very rare. In those eggs which were on the point of division when the narcotic was washed out, the splitting

of the cytoplasm took place so soon that the centrosomes did not have time to shift their positions in order to take up the position for the equatorial division. But there was enough shifting to throw the micromeral zone into one blastomere. In those eggs where division was more delayed, the centrosomes could shift their positions, more or less, and as a matter of fact, we found the division of such eggs taking place at all angles to the original axis of the egg. This explanation leaves only one point unaccounted for, the unequal division of the blastomeres. At this time I have no explanation to offer for this, unless it be due to the presence of more yolk or similar substances in the vegetative pole of the egg.

In the monaster eggs the same explanation as offered above would hold. After the retreat of the aster to one side of the egg and the appearance of the amphiaster, the micromeral zone was not divided in all cases and hence we get one or two micromeres in the 8-cell stage. When two micromeres appeared, as in figures 7, 8 and 9, we may infer that the micromeral zone was divided in two. When only one micromere appeared, we may conclude that the first division was not meridional and so the micromeral substance went to one blastomere.

In the case of the pseudotetraster eggs which were obtained from the phenyl urethane experiment, it seems that we have a second factor entering here, a factor which tends to obscure the tendency of the spindles to take up their position with regard to the stage of the cytoplasm. If we were to imagine the egg as homogeneous and were to place two spindles in it, then we should expect that these would stand at right angles to each other, since it is only in this position that the four asters exert their maximum influence. This is the position of the two spindles found in the pseudotetraster eggs and is explicable on the assumption that if a structure was present in the egg protoplasm at this time (the controls were in the 8-cell stage) it was not sufficiently impressed or not strong enough to keep the asters from taking a position where they would exert their maximum influence. As a result, we find the pigment band cut at all possible angles, as shown in figures 47 to 50. In the following division, the micromere appeared from the

blastomere which contained the micromeral zone. Again, it is worthy of note that these small cells always came from one of the large blastomeres.

In such cases as shown in figures 12 and 13, we failed to get the appearance of the micromere in the 8-cell stage, although the controls were in or past the 16-cell stage; nor did these small bodies appear in the 16-cell stage of the treated eggs. Here, apparently, there has been a suppression of the micromeres. The cause of this is not known, but it might be that the micromere formation phase of the cytoplasm, lasts only for a short time and that once it is passed over, they never form again. The development of the normal egg would seem to lend support to this idea.

What is the evidence that the small cells which appeared in these experiments are true micromeres? The most convincing point is the fact that the small cells in the treated eggs appear simultaneously with the appearance of the micromeres in the 16-cell stage of the controls. This coincidence of appearance has been sufficiently emphasised in the present paper. The further fact that once these small cells have appeared early no more micromeres are formed, is additional evidence of their nature. A third point, of lesser importance, perhaps, is that these small cells never contain pigment. These three reasons are sufficient to prove that the small cells produced early are true micromeres.

From the standpoint of embryology, the experiments recorded above are of considerable interest. The question has often been asked, does differentiation depend upon the nucleus or upon the cleavage process? As Morgan ('10) expresses it, "Do the progressive stages in the direction of specification take place during cleavage, or is the specification a process that is independent of the cleavage process? At the present time this is one of the most vital questions of experimental zoology" (p. 217).

The 16-cell stage in the normal sea-urchin egg is a differential cleavage, in the sense that at this time organ-forming substances are separated. The fact that after the removal of the micromeres the egg develops normally no more disproves that these were differentiated, than the fact that when the tentacular

region of hydra is cut off a new one grows out, disproves that the region was not differentiated, as Morgan ('96) has pointed out. Since the micromeres, as I have shown, may be formed at any time in the cleavage up to the 16-cell stage, it is evident that the process of differentiation, at least as far as the formation of the micromeres is concerned in the sea-urchin egg, is wholly a cytoplasmic affair and has nothing to do with cleavage or directly with the nucleus.

From the standpoint of the mechanics of cell-division, the present work shows that other factors are at work in the egg besides simple mechanical causes; that there is in the egg an invisible structure which seems to undergo changes and that these changes control the positions which the spindles will take in the egg and in its blastomeres.

SUMMARY

1. Monaster eggs, produced by shaking the eggs of *Strongylocentrotus*, were isolated and followed through cleavage.

2. At the time when the control eggs were in the 16-cell stage, one or more small cells appeared in the cleaving monaster eggs. The latter were usually in the 8-cell stage at this time.

3. In order to determine the cause of micromere formation, eggs were treated with potassium cyanide, and phenyl urethane, or were kept at a low temperature after fertilization.

4. By treating the eggs with phenyl urethane it was possible to throw the micromere formation in the 2-, 4- or 8-cell stage.

5. On the basis of the experimental work it is concluded that: (a) at the time of fertilization progressive changes are initiated in the cytoplasm of the egg which go forward independently of the nucleus and of the cleavage process; and (b) differentiation, as far as the formation of the micromere is concerned in the sea-urchin egg, is dependent upon cytoplasmic oxidation, the nucleus and the cleavage process playing no direct part here.

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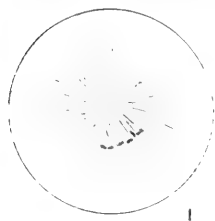
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PLATE 1

EXPLANATION OF FIGURES

1 to 15 All the drawings, except figure 1, have been made from free-hand sketches.

- 1 Schematic sketch of a monaster egg.
- 2 Egg showing protoplasmic movement.
- 3 First cleavage showing protoplasmic movements in division plane.
- 4 Two-cell stage of monaster egg with protoplasmic balls.
- 5 Two-cell stage of monaster egg.
- 6 Four-cell stage of monaster egg.
- 7 to 9 Eight-cell stages of monaster eggs showing two micromeres.
- 10 and 11 Eight-cell stages of monaster egg showing one micromere.
- 12 and 13 Eight-cell stage of monaster egg showing no micromeres.
- 14 Monaster egg with aster.
- 15 Monaster egg with aster.



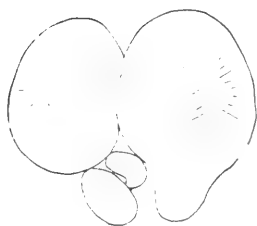
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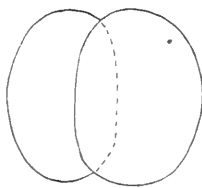
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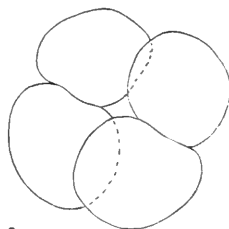
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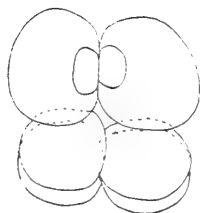
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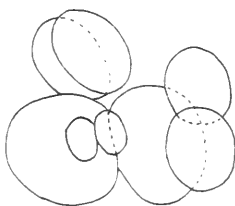
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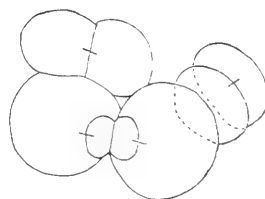
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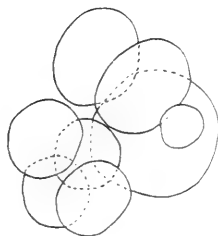
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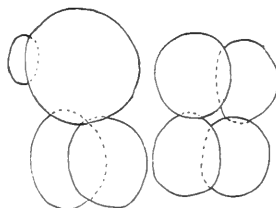
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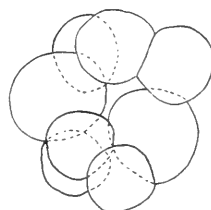
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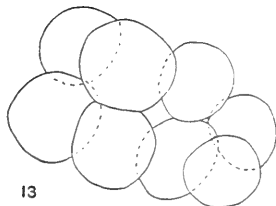
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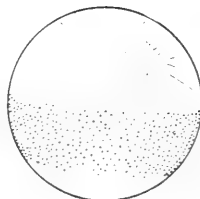
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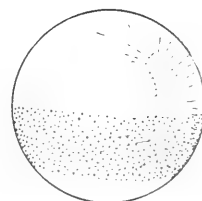
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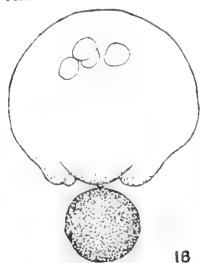


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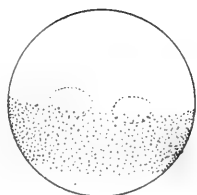
PLATE 2

EXPLANATION OF FIGURES

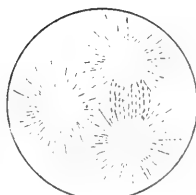
- 16 to 34 All drawings from free-hand sketches.
- 16 Monaster egg with protoplasmic ball thrown off.
- 17 Egg after treatment with phenyl urethane, two nuclei present.
- 18 Pseudotetraster egg.
- 19 Pseudotetraster egg after division.
- 20 to 22 Two-, four- and eight-cell stages of the same egg.
- 23 to 25 Two-, four- and eight-cell stages of egg treated with phenyl urethane.
- 26 to 28 Same as above.
- 29 to 31 Same as above.
- 32 to 34 Cleavage of egg treated with phenyl urethane. Two micromeres were produced in the four-cell stage.



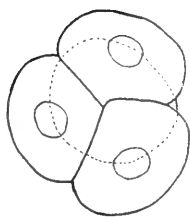
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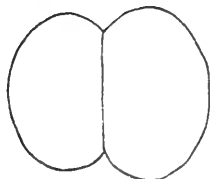
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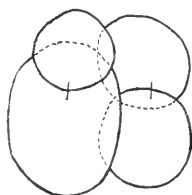
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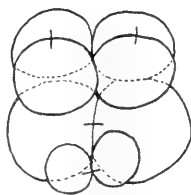
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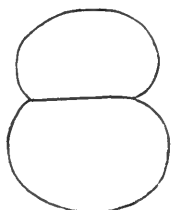
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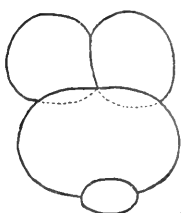
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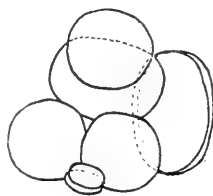
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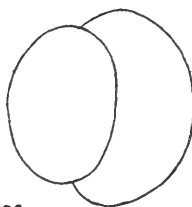
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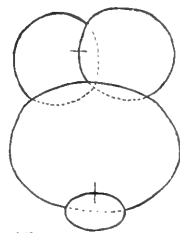
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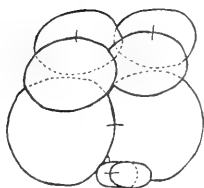
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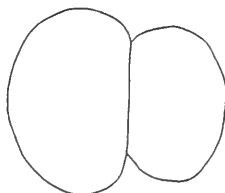
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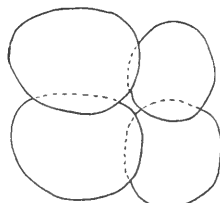
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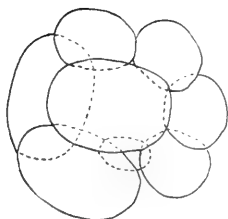
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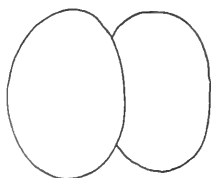
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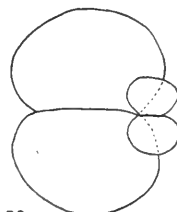
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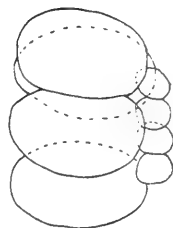
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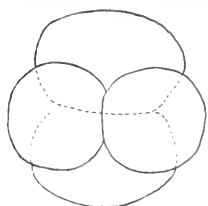


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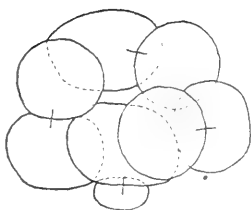
PLATE 3

EXPLANATION OF FIGURES

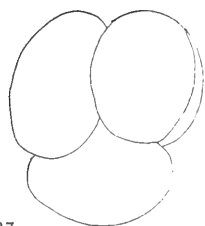
- 35 to 50 All drawings from free-hand sketches.
- 35 and 36 Cleavage of pseudotetraster egg.
- 37 and 38 Cleavage of pseudotetraster egg.
- 39 to 41 Cleavage of pseudotetraster egg.
- 42 and 43 Eggs in which the micromeres appeared in the two-cell stage.
- 44 and 45 Showing plane of division of eggs treated with phenyl urethane.
- 46 Showing position of nuclei in egg where the nucleus had divided.
- 47 to 50 Showing the planes in which the eggs were cut by the division of the pseudotetrasters.



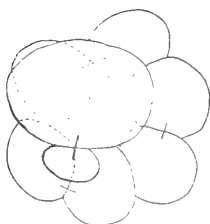
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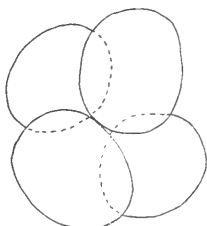
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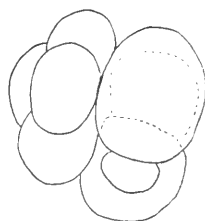
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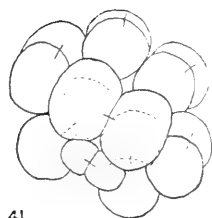
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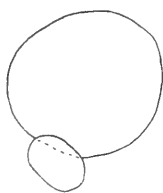
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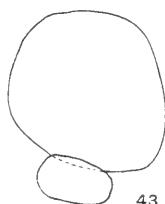
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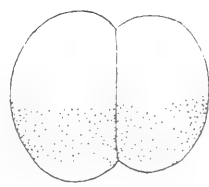
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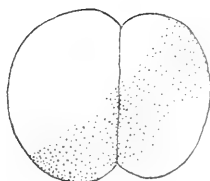
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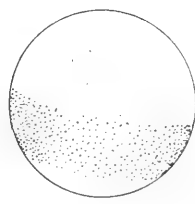
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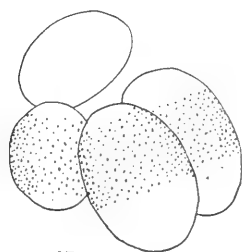
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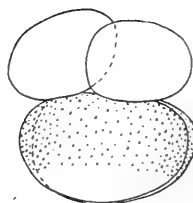
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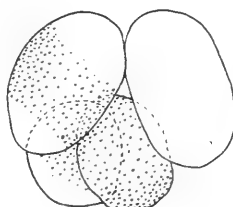
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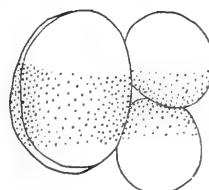
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SOME STUDIES OF ENVIRONMENTAL INFLUENCE, HEREDITY, CORRELATION AND GROWTH, IN THE WHITE MOUSE

FRANCIS B. SUMNER

SEVENTEEN FIGURES

INTRODUCTION

The present paper constitutes a final report upon a series of experiments commenced in the fall of 1906, at Woods Hole, Massachusetts. The results of the work of the first three years have been rather fully presented in several previous papers (Sumner '09, '10 a, '10 b, '11). These experiments were continued, however, until October, 1911, when circumstances compelled their abandonment. During the last of these years, a much larger number of animals were employed than previously, and a more complete series of measurements was obtained from each. Accordingly, the results which I now am able to offer are in some respects more convincing statistically than those comprised in my earlier accounts.

In this final report, the chief stress will be laid upon my later, unpublished work. I have, however, applied to some of the earlier data certain biometric methods which I did not at first attempt to employ. Furthermore, I have in cases where this procedure seemed justifiable, combined the earlier and later results, giving cumulative probability to certain conclusions.

The completion of the last year's experiments was rendered possible by two successive grants, aggregating four hundred dollars, from the Bache Fund of the National Academy of Sciences. I take pleasure, herewith, in expressing my obligation to the trustees of that fund for this important assistance. My acknowledgments are also due to the United States Bureau of

Fisheries for permission to occupy certain rooms at its Woods Hole Station, during the winter months of several years, and to Prof. F. R. Lillie, Director of the Marine Biological Laboratory at Woods Hole, for allowing me the extensive use of two rooms in one of the buildings of that institution. Without these privileges the work would have been quite impossible.

The time-consuming task of computing the data, according to statistical methods, has, in large measure, been carried on under the auspices of the Scripps Institution of the University of California, which has likewise endorsed a project by which the writer is enabled to continue studies along somewhat similar lines. To the California Museum of Vertebrate Zoölogy, through its director, Dr. Joseph Grinnell, I owe the privilege of occupying a research room in the Museum during the several months which have been devoted to preparing these results for publication.

Finally, I must acknowledge the valuable advice of Dr. Raymond Pearl, of the Agricultural Experiment Station at Orono, Maine, and of Messrs. G. F. McEwen and E. L. Michael, of the Scripps Institution, all of whom I have freely consulted regarding biometric methods. It is not my intention, however, to shift upon the shoulders of these investigators any responsibility for most of the steps which I have taken.¹

Throughout the various experiments of the earlier years, already reported upon, measurements were made of about a thousand mice. During the experiments of 1910-1911, over 1300 animals were dealt with, many of which were measured at two periods in their lifetime.² If the data from all of these individuals could be brought to bear upon any single problem here attacked, their statistical weight would of course be very great. Unfortunately, however, the size of any series having

¹ Indeed Dr. Pearl, who has been so good as to examine the manuscript of this paper, is convinced of the futility of certain of the current statistical methods which I have thought fit to employ. This disagreement springs, however, from certain fundamental differences of biological interpretation, and I am prepared to defend the stand which I have taken.

² This number does not include certain series of mice (C_1 and B_2) which were not measured, though many of them were used for temperature tests.

strictly comparable history was very much less than might be inferred from the above figures. Although it was my expectation to deal with much greater numbers of animals, particularly in the F_1 lots, the reproductive capacity of the stock proved to be seriously weakened by the conditions of the experiment or otherwise. Indeed, this unexplainable, and therefore uncontrollable factor proved to be the chief obstacle to the full success of my experiments, and is accountable for some of the obvious gaps in my evidence. For these reasons, the statistical significance of my results must rest, in most cases, on the cumulative probability derived from a number of comparatively small series.

The problem upon which it has been my chief object to throw light is the effect upon the offspring of modifications individually acquired by the parents as a result of external agencies. As is familiar to most biologists, the many known cases in which such effects have been demonstrated are commonly interpreted as due to the immediate influence upon the germ-cells of the original stimulus which caused the changes in the parent body, and not to any secondary influence exerted by the modified parts of the latter. In these experiments, I acted on the belief that a study of the structural changes, resulting from the effects of heat upon mammals, would in large measure avoid the possibility of such an interpretation, since the Mammalia are known to be homothermous, or of constant body temperature, irrespective of the temperature of the atmosphere. Certain tests of the body temperature of the mice used in my experiments have already been reported upon (Sumner '13), and will be referred to again in this paper.

The chief results of my earlier experiments, in so far as they relate to the modification of the parent generation, were published in 1909; the findings regarding the offspring were published in 1910 and 1911. The principal facts presented in these earlier papers may be summarized as follows:—

I. Parent generation. (Reared from birth in the cold- and warm-rooms respectively): (1) The tail length of the warm-room series was considerably (often strikingly) greater than

that of the cold-room series. (2) Foot length was likewise unmistakably modified in the same direction. (3) Ear length appeared to be affected in the same direction in some series, though the result was inconstant and not statistically certain. (4) In one comparatively small group, in which the test was made, the amount of hair was appreciably greater for the cold-room individuals. (5) The differences thus artificially produced were ones which have long been believed by systematists to distinguish northern from southern races of mammals. (6) During subsequent growth, these initially produced differences, at least as regards tail length, were found to diminish considerably, even while the external causal agencies continued to act with undiminished force.

II. Second (F_1) generation (those of cold-room and warm-room parentage being born and reared in a common room): (1) The tail, foot and ear length were greater among those of warm-room parentage; (hair determinations were not made). (2) As regards tail and foot length, these differences were much smaller in the F_1 generation than in the generation immediately subject to the temperature differences.

In my previous papers, I have discussed rather at length the various interpretations which might be offered for these differences in the second generation. At no time have I declared my results to be proof of, or even evidence for, the "inheritance of acquired characters." Indeed, I have insisted that in the present state of our problems this latter expression has become hopelessly obsolete. As regards the various possible interpretations of my own results, I have always expressed indecision.

I will anticipate the outcome of my later studies by stating that they have, in the main, strongly confirmed my earlier findings. Tail and foot lengths have once more been found to be modified constantly and considerably through the influence of external temperature, and in the same direction as shown by my previous observations. On the other hand, no certain modifications in ear length has appeared during my later year's work. The quantity of hair has again been found

to be greater among the cold-room than among the warm-room individuals, so far as may be judged from a rather limited series of animals, though these differences were found to disappear, or indeed to be reversed, after the withdrawal of the temperature differences.

Furthermore, animals of the second (F_1) generation have been found, in the long run, to differ from one another, according to parental history, in the manner previously described. These differences relate, as before, to tail, foot, and ear. They also relate to relative body weight, a point which was clearly shown in my earlier experiments, though I did not realize it at the time. Hair determinations, which were made in the case of one of my F_1 lots, showed an undoubted difference between the animals of cold-room and warm-room parentage, but curiously enough, it was the latter which possessed the greater amount of hair, both relatively and absolutely. It is of possible significance in this connection that these young were born some four months after the discontinuance of the temperature conditions.

Some other matters, chiefly ones relating to intra-individual and filial correlation, and to body temperature, were dealt with for the first time in this later year's work, while the subject of the rate of growth was considered in greater detail. Some of these 'by-products' of my principal research are, perhaps, equal in importance to any results which were obtained.

Since my publication, in 1910, of the first data which were obtained from an F_1 generation, my results have been discussed or mentioned in various works dealing with heredity. By Semon ('10, '12) they have been included among the data which speak for an "inheritance of acquired characters," though he does not believe the direct effect of temperature upon the germ-cells to be wholly excluded in this case. Plate ('13a), on the other hand, who likewise upholds the Lamarckian principle, does not regard as convincing evidence the results of any experiments which have not been carried beyond the F_1 generation.

Baur ('11) and Plate ('13b) emphasize the fact, to which I myself called attention, that the females of the parent generation were kept in the cold- and warm-rooms respectively until within about five days before the birth of the young. The environmental influences, they believe, acted in some way upon the animals of the second generation during their fetal life.

Other critics (e.g. Tower '12), have pointed out what they seem to regard as a serious defect in my method, namely that my cold-room and warm-room animals were not of precisely the same ancestry. In-bred stock should have been used, they seem to think, and care taken that offspring of the same parents were represented equally in the two lots.

It may be well for me to consider briefly these various objections to the relevance of my past results. As regards the possible direct effect of temperature, as such, upon the germ-cells, I have lately published ('13) the data from a considerable series of tests upon the body temperature of the mice used in my experiments. It suffices here to say that any differences in the body temperature of adults, due to even the greatest temperature differences to which my mice were exposed, were slight and inconstant, except in certain cases where abrupt changes were made. Indeed, it is very doubtful whether the mean difference between my warm and cold rooms was adequate to produce any measurable differences in body temperature between animals acclimatized to these atmospheres.³

In young mice, less than three weeks old, the case was, however, quite different. In conformity with the observations of previous investigators, I found the body temperature during early life to be very widely influenced by that of the atmosphere. The possibility therefore remains open that the external temperature reached and modified the germ-cells at this stage, even if not later. Indeed, I am myself disposed

³The experiments of Congdon ('12) seemed to show quite measurable differences of body temperature, resulting from even smaller atmospheric differences than those with which I dealt. I have already ('13) criticized Congdon's results and given reasons for believing that his findings were based upon inadequate methods of recording the internal temperature of the body.

to give more weight than formerly to this possibility, in view of certain quite meagre evidence furnished by my own experiments. Let no one delude himself into believing, however, that any direct effect upon the germ-cells (or their forerunners), at an early stage, would render more intelligible the parallel modification of parent and offspring, when such is found to occur. As judged by the criterion of *conceivability*, this explanation of the phenomena has no advantage over that of a specific induction exercised by the modified portions of the soma.

As regards the second of the objections mentioned above, I have, throughout these experiments, realized the importance of obtaining F_2 and even later generations. Such a continuation of my descent lines was fully planned for in the last year's work, and my failure to attain this end was a source of great disappointment. Aside from the fact that my entire series of experiments was involuntarily brought to a close before this could be accomplished, there was another serious obstacle to the rearing of an F_2 generation of any value for the purpose at hand. This was the impossibility, with the stock in hand, of my obtaining sufficient numbers to show with any statistical certainty such attenuated differences as might be expected to persist to this generation. The reasons for this failure will be stated more fully below (pp. 356-357).

But such an explanation of the shortcomings of my work does not, of course, meet the objection as to the futility of basing conclusions upon an F_1 generation alone. It seems to me, however, that the rearing of a satisfactory F_2 generation would not carry us much farther toward the solution of our main problems, and this whether the results proved to be positive or negative. For even the unmistakable persistence of any induced peculiarity into this generation would not, in itself, furnish evidence for the inheritance of a modification which was originally purely somatic. It would be perfectly consistent with the view that the stimulus employed evoked a germinal change at the outset. On the other hand, the disappearance of all appreciable traces of the modification in the F_2 generation would be no disproof that a somatic modi-

fication had actually been transmitted as far as the F_1 generation. Indeed, as regards the influence of some stimulus acting during a single lifetime, such a rapid attenuation of its effects would be the result most in harmony with a belief in the Lamarckian principle as generally understood. For we ought not to expect the accumulated effects of environmental influences, acting during a great number of preceding generations to be suddenly and permanently effaced by influences acting during a single generation.

In opposition to the view of Baur and Plate that the persistence of the modifications in my young mice may have been due to some effect of temperature acting upon them during fetal life, I can fortunately offer the best possible kind of rebuttal. This is the fact that all three of the F_1 lots in my later experiments (' C_2 ' ' C_3 ' and ' C_4 ,' according to my notation), were *conceived and born after the discontinuance of the temperature differences*. Indeed the ' C_4 ' lot was born nearly five months after the parents had been brought into a common room. Nevertheless, these mice, in the aggregate, show mean differences quite as great as those shown in the earlier experiment, where the mothers remained subject to the temperature differences during the first two weeks of pregnancy.

Such criticism of my methods as is based upon my failure to use animals of identical ancestry for the warm-room and cold-room sections of my parent generations appears to me to have little force. It rests on the possibility of an unconscious choice of individuals for the two parent lots belonging to "bio-types" having mean heredity differences of just the sort which I was later able to detect in the offspring. In such an event, the differences in the latter might bear no relation whatever to the treatment to which the parents had been subjected. It would, of course, be possible so to select two groups of mice that the mean foot or tail length of their offspring would be materially different. But happily we are enabled to state with some accuracy the degree of selection which is necessary in order to bring about a result of a given magnitude. I shall show below, by well-known statistical methods that differences of the

magnitude which I have found to occur between the mean tail length of my cold-room and warm-room descendants could only be accounted for by the selection of parents displaying an average difference of many millimeters. That such a selection was actually made will be shown to be a practical impossibility.

In concluding this introductory section, I will refer to a recent piece of work, somewhat similar to my own in its general bearings. I refer to the experiments of Przibram ('09) upon rats, the results of which have unfortunately never been very fully reported. This investigator subjected rats to widely different temperatures⁴ for several generations. Certain differences between the two lots appeared, as a result of this treatment, notably a hypertrophy of the testicles and a diminution in the quantity of hair in the warm-room animals, as compared with those reared in the 'normal' room. So far as may be judged from Przibram's single brief report upon his work, no measurements were made, and only such conspicuous differences were considered as could readily be observed with the unaided eye. Przibram found that both of these peculiarities of his warm-room rats disappeared, *within the lifetime of the individuals*, when the latter were removed to normal temperatures. Now if rats of the second or third experimental generation were removed from the warm-room, and were paired, even before these induced modifications had disappeared, the modifications were not detected in the offspring. If, however, they were paired *before* removal from the warm-room, so that fertilization and early pregnancy occurred in the latter, the parental modifications did reappear in the next generation. Although present to a diminished extent, the differences were great enough to be detected by the unaided eye. It is not stated whether the rearing of an F₂ generation was attempted.

While these experiments of Przibram's are of decided interest, it seems to me that their significance has been over-rated by some writers. In the first place, the alterations in the

⁴ The warm-room lot were kept at 30 to 35°C., the others at 'normal' temperatures.

parent generation appear to have been functional, rather than structural, of if the latter, they were, at least, not permanent. Differences in the activity of the hair follicles, and in the degree of relaxation of the scrotal sack may have been wholly responsible for the visible phenomena. As already stated, I am able to confirm Przibram's observations as regards the disappearance of the differences in the quantity of hair, after the withdrawal of the effective stimuli. But in my own experiments, both the original differences and their subsequent disappearance (or even reversal) were not obvious to the eye, but were detected only by the careful gravimetric treatment of a considerable number of pelages. I have, however, observed a conspicuous diminution of hair in several broods of mice, belonging to a second generation which was reared in the warm-room, though the meaning of this evidence is rendered uncertain by the almost pathological character of this particular lot of young mice, and by the fact that a number of scantily clad individuals also appeared in the cold-room.

I am likewise able to confirm Przibram's observations regarding the (apparent) enlargement of the testes in individuals kept in a warm-room, though I have been disposed to regard this as due to the relaxation of the scrotal sack, that is to a variation in muscular tonus, and not to a structural change at all. Nevertheless, even if they are purely 'functional' in the medical sense of the term, these differences certainly have some material basis, and their reappearance in the offspring is a fact of considerable interest.

The circumstances under which Przibram was able to effect a repetition of these modifications in the offspring resemble those which obtained during my experiment of 1909. The modified parents were paired in the rooms of differing temperature, and were kept there during the earlier stages of pregnancy. As stated above, I later found equally great differences when the temperature conditions were withdrawn long before the pairing.

I will not here attempt to discuss the numerous and important papers of Kammerer, who has attacked the same general

set of problems by diverse methods and with a considerable variety of animals. These and various other recent studies in this field of research have lately been admirably summarized by Semon ('12) and have been discussed critically but less fully by Plate ('13).

EXPERIMENTAL METHODS

The methods which were followed throughout these experiments have already been described in some detail ('09), so that I shall content myself here with a briefer account of them. Some changes have, however, been introduced during my later studies, particularly in the statistical treatment of results.

Of the two rooms used, one (the 'cold' one) was situated upon the upper floor of an unheated building, and freely ventilated through at least two open windows. The 'warm'-room was heated by a large steam radiator. It is obvious, therefore, that the proper temperature conditions for the two rooms could be maintained only during the colder months of the year, and accordingly this phase of the experiments was limited each year to the months of November to March or April, inclusive. Unfortunately my control of temperature conditions was at no time adequate. Spells of warm weather occasionally gave to the cold-room a temperate atmosphere, even in mid-winter, while extremely cold, windy spells in a few instances reduced the temperature of the 'warm'-room to a point nearly as low as the average condition of the other one. Such conditions were very exceptional, however.

During the season of 1910-1911, the mean temperatures of the cold- and warm-rooms, based upon continuous thermograph records, were 4.2 and 22.5°C., respectively (39.5 and 72.5°F.). These figures relate to the entire period from the birth of the first experimental generation to the discontinuance of the temperature differences. The mean temperatures during various phases of the experiment, as well as maximum and minimum figures, and mean daily ranges, are given in table 1. The seasonal temperature changes, based upon weekly means, have been plotted in figure 1.

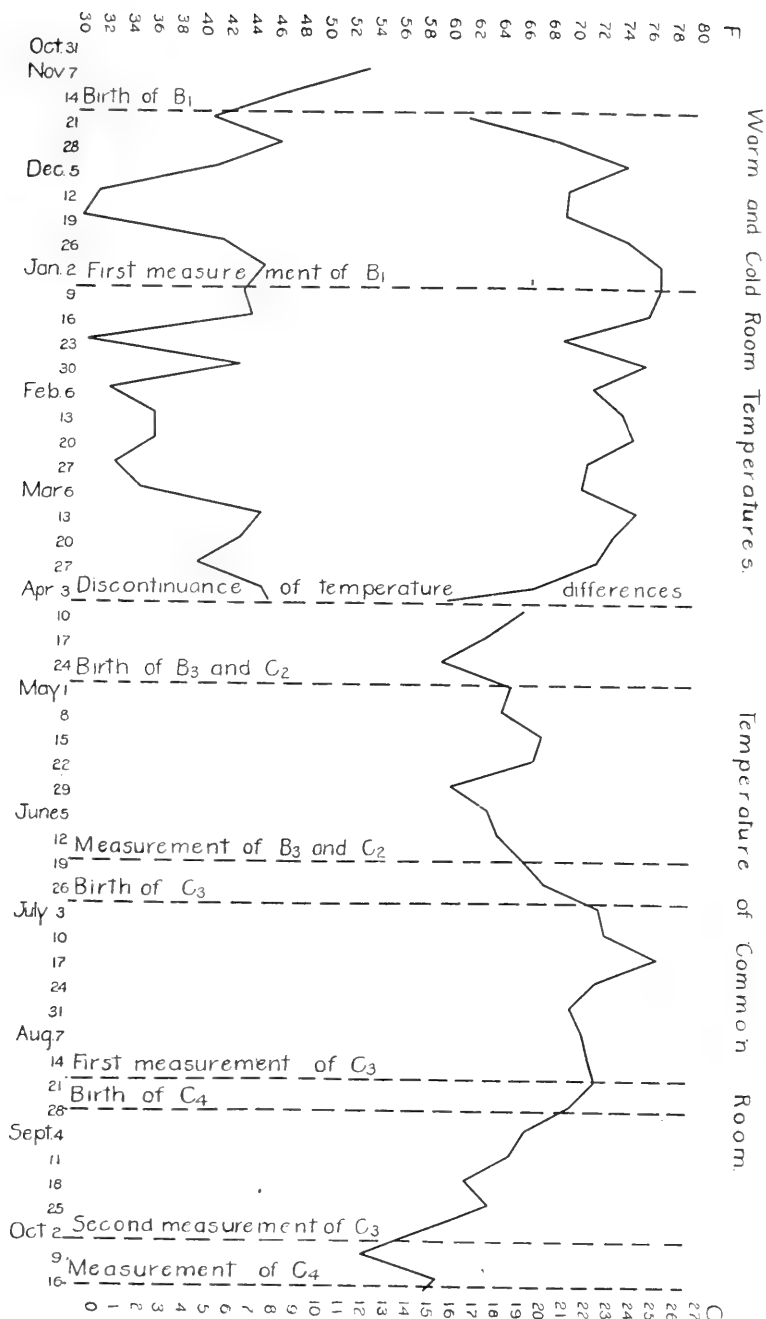


Figure 1

It is unfortunate that more exact data regarding humidity cannot be given. A suitable instrument for making these determinations was not obtained until the latter part of the experiment. A limited number of records which were made with an ordinary wet-and-dry-bulb thermometer, indicated relative humidities of about 76 and 38 per cent, respectively, for the cold- and warm-rooms. In as much as the psychrometer used was stationary, these figures, particularly that for the warm-room, are doubtless too high.⁵

However desirable for the sake of formal completeness, it is doubtful whether an exact series of hygrographic observations would have materially increased the value of the present studies. The important fact to bear in mind is that my two rooms were characterized by great differences in relative humidity, as well as in temperature, and that, so far as we know, the former may have been quite as effective as the latter in producing the structural modifications in the animals.

At a certain time in the course of the experiments (April 4, 1911), the temperature differences, already considerably diminished, were discontinued entirely. Thereafter, the animals, regardless of history, were kept together in the attic of a small building, where the temperature, throughout the summer, approached much more nearly that of the warm-room than that of the cold (fig. 1). Care was taken that the offspring of warm-room and cold-room parents should be subjected, as far as possible, to identical temperature conditions. The two sets of cages were kept upon alternate shelves, one above the other.

The same system of measurements was employed here as in my earlier studies. The data recorded were (1) weight, (2) body length (from snout to anus), (3) tail length (anus to tip), (4) foot length (from heel to tip of middle claw), (5) ear length (from 'notch' to tip), (6) weight of hair, after removal from skin.

⁵ Since very little compensatory evaporation occurred in the warm-room, the absolute amount of moisture in the atmosphere probably differed little from that of the cold-room. Air having a relative humidity of 75 per cent at 4.2°C., when raised to a temperature of 22.5°, would have a humidity of less than 25 per cent.

In view of the comparatively slight differences with which we are frequently dealing, the value of such observations as the present ones is obviously dependent upon the care with which the measurements are made, and particularly upon the complete comparability of the various figures for a given part throughout the entire series of experiments. The system of measurements earlier developed by me has therefore been adhered to rigidly, the animals and the instruments being, so far as possible, manipulated in the same manner throughout.⁶ During the last year's experiments a special dial caliper was constructed for this purpose.⁷ Each measurement was taken twice, and the dial or scale of the instrument was so held as to face away from me, in order that the reading might not be known until the measurement was completed. As an illustration of the precision which I found it possible to reach, it may be stated that for foot length the mean difference between the first and second reading of the caliper was about 0.14 mm., while for ear length it was only 0.06 mm.⁸ And yet in no case were the two measurements of the same part made in succession, but those of different parts alternated with one another.

In dealing with animals of the second generation (offspring of modified parents), in which the mean differences between two contrasted lots were small in comparison with their variability, it was necessary to guard against any possible unconscious bias in making the caliper measurements. I therefore continued the practice, already adopted for my 1909 series, of keeping myself in ignorance of the parentage of a given mouse until the measurement was made ('10 b).

⁶ See my paper of 1909, pp. 101-105, for an account of this system.

⁷ This was made by the B. C. Ames Co., of Waltham, Mass. Arthur Meissner, of Freiberg, i. Sa., Germany, now has on the market a dial caliper which appears to be quite satisfactory. This type of caliper was used by me only for the smaller measurements (foot and ear). For body and tail length, which were recorded to the nearest millimeter, an ordinary sliding caliper was preferred.

⁸ Individual readings were recorded to tenths of a millimeter. In a considerable proportion of cases this difference was 0.

In measuring certain series of mice, the latter were killed. But this was not possible in many cases, for the animals were needed for further experiments. The measurements of tail length which were made upon living mice were not quite comparable with those made upon dead ones, the latter giving a length which was commonly less by one to two millimeters, depending upon the weight of the animal. In order to take the ear and foot length of living mice, it was necessary to anesthetize them with ether or chloroform. For the latter measurement, the sole of the foot was pressed against a flat surface by means of small forceps, instead of being impaled by a pin as in the case of dead animals. It was not found practicable to determine the body length of living mice, even when anesthetized, since it was necessary to pin out the animals to accomplish this. Hence the method of grouping according to body length, in comparing two series of animals, was not practicable with those which were measured when living.

The technique employed in the determination of hair weight has been described in a paper already referred to ('09). The hair was removed from each pelt, chemically cleaned and thoroughly desiccated, before being weighed. In the case of my 'C₄' lot of mice in 1911 (p. 385), I was forced to adopt a variation in the procedure. I did not have time to skin the 210 individuals, as had been done for all of the previous sets, and it was consequently necessary to preserve the bodies entire, pending the resumption of the work. Since ordinary preserving reagents (alcohol or formalin) would have fixed the skins and prevented the easy removal of the hair, the bodies were kept in an aqueous solution of thymol. It was found that in this fluid the skins underwent maceration to a considerable extent, so that the hair was easily and completely removed by forceps. The hair weights for this series of mice are perhaps not wholly comparable with those for the skins which were treated with lime-water, but the important comparison—that between the warm-room and cold-room sections of the same series—is perfectly valid. The task of preparing and weighing the hair from the nearly 400 pelts which I preserved in 1911 was carried out by Dr. G. F. White, of Clark

University. The weights of the individual pelages were ascertained to the nearest one-tenth milligram, though this degree of exactness was doubtless superfluous.

Every mouse used in the present work was given an individual number and identification mark, and record was kept of the parentage of every animal born.

STATISTICAL METHODS²

In the present paper I have computed the averages for all of the series of measurements and the standard deviations for the most important ones. The probable errors of the averages have been given in cases where the significance of the figures has been materially enhanced thereby.

In any investigations, such as the present ones, in which two series of individuals are compared which are suspected of differing in the characters measured, a point of vital importance is the significance of any differences which may be found between two averages. The mere fact that in series *a* the mean tail length is found to be greater than in series *b* is no indication that this difference is relevant to the inquiries in hand. This is true for two reasons. In the first place, the difference may be 'accidental,' that is, due to a multitude of independent causes having no essential relation to the conditions of the experiment. The odds against such an interpretation may readily be calculated from the relative magnitude of the difference between the two averages in question and the probable error of this difference. The latter is expressed by the formula

$$\sqrt{E_1^2 + E_2^2}$$

² In my paper of 1909, a few pages were devoted to an exposition of some of the most elementary principles of statistical treatment. For very much more extended and technical discussions of the subject, the reader is referred to C. B. Davenport ('04), E. Davenport ('07), Pearson ('00), Thorndike ('04), Yule ('11) and various others. Unfortunately, the non-mathematical student who desires to do anything beyond obtaining the simplest of the biometric constants is confronted by rather formidable difficulties. And the plight of the experimentalist is rendered still worse by the fact that his special requirements have seldom been kept in view by the statistical experts.

i.e., the square root of the sum of the squares of the probable errors of the respective averages. The likelihood that a difference of a given sign is significant, i.e., not due wholly to 'chance,' depends upon the ratio which it bears to its probable error. The chances are only 3 out of 4 when the difference equals its probable error. They are 997 out of 1000, when the former is four times the latter.¹⁰

But there is another reason why even a considerable difference between the mean values of a given character in two series of animals would not necessarily have any relevance to our inquiries. This is the fact that the animals of the two series might differ widely in their mean size. The discovery that the tail length averaged 10 per cent greater in one group than in another would be of little interest, if it were found that the body length was likewise 10 per cent greater. *No comparison between two groups of individuals, as regards the mean absolute magnitude of any part or organ, is commonly justified unless the average size of body is approximately equal in the two groups.*

The foregoing proposition, which seems self-evident and superfluous, has none the less been largely ignored by some of those who have dealt most widely with animal measurements. In the works of systematic mammalogists, for example, we find pages devoted to the absolute measurements of certain parts, and to comparisons between their mean values in related species, when these species differ obviously in their average bodily size.¹¹ This procedure is of course instructive in case the parts in question differ so widely in their relative magnitude in the two species that the distinction is obvious without reference to the size of the individuals compared. And this is not infrequently true. Furthermore, it must be con-

¹⁰ I have given a brief table of such values in my paper of 1909 (p. 107).

¹¹ 'Body length,' in the sense in which I have used the term, is commonly not referred to by these writers. They give, instead, the 'total length,' i.e., the length of the body, plus that of the tail. But this is obviously an unsatisfactory index of size, in comparing two species which differ widely in their relative tail length. And even within the same species, there is a wide range of variability in the ratio between tail and body (p. 389).

ceded that in many cases comparison by size-groups is impracticable for the taxonomist, owing to lack of a sufficient number of specimens.

The need seems obvious of suitable methods for comparing identical structures in two groups of individuals, each presenting a wide range of bodily size, and particularly groups which differ from one another considerably in their mean size. And yet, curiously enough the standard reference works on biometry give little or no attention to this phase of the subject.

Two principal modes of procedure suggest themselves for making comparisons of this sort. The first is to transmute the absolute values into relative ones, that is into ratios, expressed preferably as percentages of the body length. Such a course is obviously only possible with those organs which increase in exact proportion to the body length, i.e., which maintain the same ratio at all stages of growth. This seems to be approximately true for tail length, as will be shown below (pp. 424-425), and the fact has rendered possible some very instructive comparisons in the case of my mice. But it is obviously not true for foot and ear length. In table 26 it is shown that the foot length of the second lot considered ('breeding males') increased only about 1 per cent between January 5, when the animals were 50 days old, and August 22, when they were 9 months old, and a consideration of the individual cases (not shown in the tables) shows that there was, indeed, in some instances an actual diminution in length. On the other hand, the weight of the animals increased, on the average, between 50 and 60 per cent, during this period. From a consideration of other cases, it may safely be inferred that this increase in weight corresponded to an increase of about 20 per cent in body length. It is, indeed, an obvious and familiar fact that the feet of many young mammals are proportionately much larger than those of the adults. The same is true to a lesser degree of the ears.¹²

¹² I do not, of course, wish to deny that foot and ear length are strongly correlated with the length of body. Larger animals have, on the average, larger feet and ears, as will appear constantly from my data to be considered later. But the growth of these appendages practically ceases long before that of the body.

The other method of comparing two series of animals, both very diverse in size, is the simple expedient of dividing each series into a number of groups, containing individuals of approximately the same size. Comparisons may then be made between the mean values for any character in corresponding size-groups of the two series. This is a method which I have already used in these studies ('10 a, '10 b) and have employed widely in the present work. The animals have most frequently been grouped according to body length, the groups containing individuals which differed by less than 2 millimeters (e.g., 80-81, 82-83, 84-85, etc.).¹³ As stated above, certain lots of the animals were measured without killing them, and the measurement of body length in living mice is scarcely practicable. In such cases, therefore, the individuals have been grouped according to weight, the groups differing in mean weight by 1 gram. This method of grouping is, however, less satisfactory than when body length is employed. As will be shown below (table 20) the correlation of tail, foot and ear length with body length is higher than with weight. Whichever mode of grouping is employed, it must be borne in mind that, in dealing with immature animals, only series of the same age should be compared by this method.

The method of comparison by size-groups is subject to one decided limitation, namely, that unless the series dealt with contains great numbers of animals, the single groups are necessarily small. At the two extremes in the scale of size, they may be constituted by only one or two individuals. Indeed, in cases where the difference in mean size of the two series of animals is considerable, the smaller animals of one series and the larger ones of the other may not be available for comparison at all. Thus, in most cases, the probable errors are large in comparison with the differences which are dealt with. We are therefore forced to rely, in estimating the probability of our results, upon the cumulative testimony of a considerable number of cases, each having a low degree of certainty. One method of calculating the probability, indeed, is to consider the chances

¹³ In the 1909 series 1-mm. groups were used.

that a certain proportion of the differences should bear the same sign. What, for example, are the chances that in twelve pairs of contrasted size-groups, the mean tail length of series *a* should be greater in ten cases than that of series *b*? Theoretically, the probabilities against such a happening being the result of mere 'chance' can readily be computed, though this method must be applied with considerable caution.¹⁴

Besides an enumeration of positive and negative cases, I have resorted to two other, more fruitful, methods of comparison by size-groups. One of these is to plot the 'curve' or 'graph' for each character in each of the two contrasted lots of animals. Body length (or weight) is expressed by the abscissae, and tail, foot or ear length, or some other magnitude, by the ordinates. Each curve connects the mean values of a given character for all the size-groups of a series, and the two contrasted lots may thus be compared very instructively.¹⁵ Such comparisons have been the object of figures 2 to 17. In many of the latter it will be seen that the curve for one lot is, throughout its course, pretty constantly above or below that for another lot, the width of the interval varying from point to point.

It is desirable to obtain a value which will express the mean width of this interval. For that purpose, a simple average

¹⁴ See my paper (1910 b, pp. 329-332). This criterion of probability was applied rather extensively in my previous reports of this work. The probabilities calculated in the way stated are somewhat deceptive, however, in that all the individuals of a series are not independently variable. Many of them are brothers or sisters of other members of the series, and the fraternal correlation is high. Again, the chances of coincidence in the sign of the various differences for tail, foot and ear, taken collectively are considerably increased by the correlation which is known to exist between these organs (pp. 400-415). Finally, in such a mere enumeration of positive and negative cases, one case is regarded as of equal weight with another, whereas the first may be based upon ten times as many individuals as the second, in which event it would have much more significance. Insufficient weight was probably given to these facts in my former studies.

¹⁵ The figures found at intervals along certain of the curves here published denote the numbers of individuals in the respective size-groups. It must be borne in mind that all parts of these curves are not of equal significance. The more central portions, which are based upon the majority of the individuals, of course count for far more than the terminal portions, which may be based upon very small numbers.

of the differences between the successive size-group means might be used, as was done in one of my earlier papers. In this case the various differences would be regarded as of equal weight in computing the average, whatever the number of individuals in the size-groups. It seems fairer, however, to weight the various differences in proportion to their significance in the series. This I have done by multiplying each difference by the product of the number of individuals upon which the two respective means are based. The sum of these weighted differences has then been divided by the sum of the weighting factors. This may be expressed by the formula:

$$M_{dif} = \frac{\sum [(m-m') \cdot (n \ n')]}{\sum (n \ n')}$$

in which m and m' are two corresponding size-group means and n and n' are the numbers of cases upon which these means are based.¹⁶

The probable error of the mean difference, as thus computed, may be expressed by the general formula for the probable error of a mean, viz.:

$$.6745 \frac{\sigma}{\sqrt{N}}$$

The standard deviation (σ) here employed would be that for the size-group differences, considered as variates, the square of each deviation from the mean difference being weighted by the factor $n \ n'$ as above, and the product-sum being divided by $\Sigma \ n \ n'$. The N in the denominator is the number of size-group differences. I have not, however, computed the probable errors for any of the mean differences given in my tables. The number of size-group differences was so small (commonly 10 or less) that the probable errors would have had little meaning. The

¹⁶ This is really very simple; $m-m'$ represents the vertical distance between any two corresponding points in a pair of curves under comparison, n and n' being the numbers indicated at these points; (see foot-note 15). The sign Σ denotes the summation of a series of homologous values.

constancy or inconstancy of these differences, as revealed by the graphs, furnishes a much better indication of the relations between these contrasted series.

I have dealt at some length with this method of comparison by size-groups, because it appears to me to be a highly important one in studies of this sort, and because it has, despite its extreme simplicity, received little or no attention in any of the familiar works on biometry.

Another matter which demands some attention at this point is that of correlation. Since this subject may not be familiar to some who could apply it very advantageously in their work, I shall preface my remarks with an elementary explanation of the correlation coefficient. The latter is a measure of the degree to which the deviation of one part from its mean value entails the deviation of some other part from its mean value. The two parts considered may be either two different organs in the same individual or identical organs in two related individuals. Indeed the same method may be applied to any two facts in nature which are suspected of varying coincidently. Pearson's well-known coefficient of correlation is expressed by the formula:

$$r = \frac{\sum (dev\ x \times dev\ y)}{n\ \sigma_x\ \sigma_y}$$

in which r is the correlation coefficient, $dev\ x$ and $dev\ y$ are the deviations of the two members of an associated pair of characters from the respective mean values of those characters in the population under consideration; n is the number of these pairs in the series; while the other two factors in the denominator are the standard deviations of the two characters.¹⁷

¹⁷ The only feature of this formula of which the reasonableness is not obvious is the inclusion of the two standard deviations in the denominator of the fraction. This is done in order that the deviations of each part shall not be treated as absolute values, but shall be expressed in terms of the variability of that part. A given deviation in one organ may be twice as great as that of another organ, but its significance is obviously not twice as great if the variability of the former part is likewise twice as great as that of the latter. The formula given above is really an abbreviated expression for:

$$r = \sum \left(\frac{dev\ x}{\sigma_x} \times \frac{dev\ y}{\sigma_y} \right)$$

In the multiplication of deviations, cases in which a pair of these have the same sign of course give a positive product, and tend to raise the value of the coefficient; cases in which they are of a different sign give a negative value and tend to lower the value of the coefficient. When the former cases outweigh the latter, the coefficient is positive, when the latter outweigh the former it is negative. The correlation coefficient may range in value from $+1$ (= perfect positive correlation) to -1 (= perfect negative correlation), 0 denoting total absence of correlation. The probability of obtaining a coefficient of exactly 0 is very small, so that low figures, either positive or negative, are to be regarded as of little significance, particularly when based upon small numbers. The probable error of the correlation coefficient is expressed by the formula:

$$E_r = \frac{0.6745 (1 - r^2)}{\sqrt{n}}$$

in which r is the coefficient and n the number of related pairs upon which the determination is based.

When based upon adequate numbers, the coefficient of correlation enables us to state with confidence the *most probable* deviation of one organ which will accompany any chosen deviation of the other, in the population with which we happen to be dealing. If, for example, the correlation between foot length and tail length is $+0.5$, the standard deviation of the former being 0.5 mm. and that of the latter 4.0 mm., a group of animals having a tail length 10 mm. above the average will, in general, have a foot length 0.625 mm. above the average.¹⁸

In my own work the degree of correlation has been computed between every two characters measured. The labor of

¹⁸ This is solved by the equation:

$$x = r \frac{\sigma_x}{\sigma_y} y$$

(see Yule, 1911, p. 171). In the present instance x represents the abmodality of foot length sought, r the coefficient of correlation between foot and tail, and y the known abmodality of tail length, while the numerator and denominator of the fraction are the standard deviations for foot and tail, respectively.

such computations was minimized by entering the deviations for every character of every animal, alongside the individual measurements, the latter having been recorded upon sheets which were ruled both horizontally and vertically. Multiplications could thus be readily effected between the proper figures in any two columns, the multiplying (and simultaneously the summing) being carried out with a computing machine.

In the study of correlation, we again encounter difficulties which spring from the diversity of our stock in respect to size. Suppose we wish to compute the correlation between tail length and foot length in a considerable series of mice. We may proceed in two quite different ways and obtain two quite different values. In the first place, we may treat our entire series of animals as a single population, compute the averages for this series, obtain the deviations from these averages, and proceed as above described. This will give us the correlation coefficients for the population as a whole, irrespective of size. It is such coefficients as these which chiefly figure in the tables given in the treatises on biometry, and I have myself computed them for certain of my series of white mice (tables 20 and 23). But it seems to me that, however accurately obtained, such figures are commonly of secondary biological interest. For, to refer again to our illustrative case, the correlation between foot length and tail length, in a general population of heterogeneous size, results largely from the fact that both are correlated with body length. That larger animals have, on the whole, longer tails and likewise longer feet is a platitude, and an exact mathematical expression for this platitude appears to the writer to be of distinctly minor importance for evolutionary science.

We may, however, ask the question: *Is a longer tail generally associated with a longer foot, among individuals of the same size?* In order to answer this question, I have determined the coefficients of correlation within the larger size-groups of certain of my series, thereafter finding the mean of these various group-coefficients. In this way I have been able to show that tail and foot length, and probably, indeed, any two characters

among those measured, are correlated in the second of these senses (tables 21 and 22).¹⁹

Besides intra-individual correlation, as above exemplified, we have inter-individual or genetic correlation, and this has been much used as an expression of the force of heredity. It seemed desirable that I should compute this for at least one character among those measured. In dealing with populations, so heterogeneous in size, it is plain that absolute measurements could not be satisfactorily employed.²⁰ For example, a long-tailed father or mother might have a brood of small offspring, possessed of relatively long, though absolutely very short tails. Neither is the method of size-groups adapted to this end. Fortunately, it was found that relative tail length (i.e., the ratio of tail length to body length) presented little, if any, constant differences throughout a series of individuals ranging widely in size. This figure was therefore used in computing my coefficients of heredity. Despite the relatively small numbers of individuals available, particularly in the parent generation, an unmistakable positive correlation was established.²¹ Here, too, considerably different figures were obtained, depending upon

¹⁹ The same correlations may, of course, be determined by the method of 'multiple correlation' (see Yule, 1911, chapter xii). This method makes possible the inclusion of all the individuals in the population dealt with, which is, of course, a distinct advantage. In dealing with size-groups, it has seemed preferable to throw out all groups containing less than ten individuals, a procedure which results in a serious reduction in the available material. On the other hand, the method of multiple correlation is difficult of application, except by a person having considerable special mathematical training. As a matter of fact, the more familiar illustrative tables, giving correlation coefficients, particularly for man, ignore this principle altogether.

²⁰ At least except for body length, and a computation of the coefficient of heredity for this character seemed hardly likely to repay the effort. That for stature in man, has, of course, been ascertained very exactly.

²¹ For reasons of practical expediency, which need not here be detailed, I have based my figures upon the correlation between each of the parents and each of the offspring, *taken individually*. Students of biometry commonly either choose one of the offspring arbitrarily from each fraternity, or take the latter itself as a unit, using the mean value of the given character for each fraternity, as a basis for correlation with the parents. My figures for these coefficients are therefore not entirely comparable with those obtained by other in-

the inclusiveness of the groups which were treated as single populations in the computations. But this phase of the subject may better be treated along with the results, rather than under the head of methods.

In concluding this brief statement regarding correlation, it is only fair to remark that the subject is beset with pitfalls, and that the existence of even a large and statistically reliable coefficient does not invariably imply a biological fact of any real significance. The investigator must continually be on his guard against these errors of interpretation (pp. 409-420).

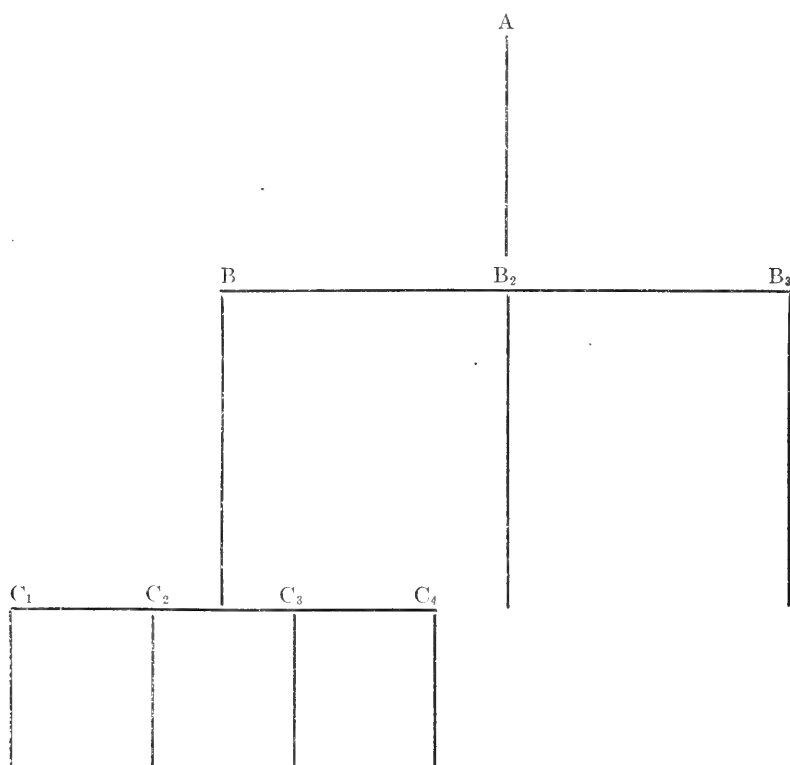
GENERAL HISTORY OF THE STOCK

In table 2 are represented the relationships among the various lots of mice employed in the present experiments, and briefly the disposition of each lot. The generation designated by the letter A represents the original stock of adult, or nearly adult animals which became the parents of the first experimental generation (B_1), as well as of two other lots (B_2 and B_3), which were not used for further breeding experiments. The males of the A generation were 4 to 5 months old when received, the females being 5 to 7 months old.

This original stock, consisting at the outset of nearly 250 mice, were divided into two portions, destined to become the parents of the warm- and cold-room sections, respectively, of the generation to be modified through temperature. In making this partition of the 'A' mice, the two lots were systematically divided so that the average weight and tail length might be approximately equal in the two. I attach very little importance to such a partition of the stock, however, since by no means all of these mice became parents, and the mean characters of those which did might not have been identical in the

investigators. It will be seen that the method which I have employed virtually weights the results on the basis of parental fertility. In a sufficiently large series, however, the coefficients obtained in these different ways would be practically the same, except in the event that the correlation tended to be constantly greater or less for the larger broods than the smaller ones. We have no reason for believing such a condition to obtain.

TABLE 2

Generations of mice employed in experiments of 1910-1911

A Original lot, received October, 1910 (49 males, 197 females), supplemented by 27 males, received January, 1911. After the birth of the first offspring (B_1), these mice were divided into two lots and placed in the same rooms (warm and cold) as their young.

B_1 First offspring of A lot; median date of birth November 16, 1910. Number reaching age of 50 days (time of first measurements), 322. Of these, 142 were reared in the cold-room, 180 in the warm-room. Of the warm-room lot, 122, constituting the 'a' section were transferred immediately after birth to the warm-room; 58, constituting the 'b' section, were first kept for 2 weeks in the cold-room.

B_2 Second offspring of A lot; born March, 1911; number of these so small and mortality so high that they were not saved for further breeding experiments.

B_3 Third offspring of A lot; median date of birth April 27, 1911; number reared to the age of 50 days, 157. Of these, 107 were born of parents which, after reaching maturity, had been kept 3 to 5 months in the cold-room, while 50 were born of parents which had been kept for the same period in the warm-room.

TABLE 2—Continued

C₁ First offspring of B₁; they were born at the same time as B₂, and for the same reasons were not measured nor kept for breeding experiments.

C₂ Second offspring of B₁; median date of birth April 27, 1911; number which were reared to age of 50 days, 159. Of these, 94 were born of parents which had been reared from birth in the cold-room, 65 being born of parents which had been reared in the warm-room. The C₂ mice were, however, conceived and reared in a common room, the temperature differences having been discontinued at the time of the mating of the parents.

C₃ Third offspring of B₁; median date of birth June 28, 1911, or 12 weeks after discontinuance of temperature differences. The number born of cold-room parents, which reached the age of 50 days, were 120, the number of warm-room parentage being 104.

C₄ Fourth offspring of B₁; median date of birth August 26, 1911, or nearly 5 months after discontinuance of temperature differences. Number of cold-room parentage reaching age of 50 days, 118; number of warm-room parentage, 93.

two sections.²² Furthermore, only weight and tail length were considered in this connection.

The B₁ mice, which underwent divergent modification as the result of temperature differences, constituted the parent generation, as the term is ordinarily employed in breeding experiments, the four successive broods of 'C' mice representing the first filial generation ('F₁' in the current phraseology). Of the B₁ mice, there were born in all 121 litters. Of these 31 entire litters died early in life. A still further reduction occurred through the death of many individual members of surviving litters. That any selective elimination resulted from this mortality, of a sort which accounted, in any degree, for the differences which were later found to exist between the two contrasted lots of mice, or their offspring, might, of course, be maintained as an academic proposition, but I think there is nothing to justify it. The mortality occurred in large part during intra-uterine life, the young being born dead, before the mothers were transferred to separate rooms. And a large part of the remaining deaths were due to the young being born in an enfeebled condition, not fitted for survival in any environment. As regards the greater part of the mortality, therefore, it is impossible to see how selection could have operated

²² As a matter of fact, they agreed pretty closely, as will appear from table 15.

in the way suggested. It could conceivably have done so, however, in the case of such animals as died during adolescence, though the possibility that any of the results here to be described are thus accounted for is, I think, very remote.

During my first two years' experiments of this sort (those of 1906 and 1907) I adopted the plan of dividing each litter belonging to the B generation into cold-room and warm-room halves respectively. Thus the two sections of this lot of mice would agree more nearly in their mean hereditary characters than if they had been taken at random. This procedure I discontinued as not worth the additional trouble involved. To have the warm- and cold-room lots agree in their mean hereditary composition (or rather, in their parentage, which is quite a different thing) would be of avail only if all or most of these animals became parents. Otherwise, the two lots to be compared in the next generation might differ considerably in ancestry. The question whether the structural differences which were actually found could have been due to such differences in ancestry has been referred to above (p. 332) and will be considered again later.

In table 2 the fact is noted that the warm-room section of the B₁ lot of mice was divided into two portions ('a' and 'b'), one of which was transferred to the warm-room, immediately after birth, the other being first kept for two weeks in the cold-room. This was done in order to test the question whether the germ-cells might not be directly influenced by temperature during that earlier period of life when the temperature of the body varied widely with that of the atmosphere.²³ Unfortunately, the number of individuals in the division ('b'), which was kept for a time in the cold-room, was not sufficiently great to insure an adequate number of progeny. The unsatisfactory, though interesting, data from this experiment are discussed below (pp. 402-404).

Since, throughout my breeding experiments, one male was commonly mated with several females, a considerable proportion of the B₁ males were killed and measured at the age of four

²³ Vide Sumner, 1913, pp. 344-351.

months. In selecting the males to be used for breeding purposes, the largest (heaviest) member of each litter was taken, the others being relegated to the group of 'superfluous males' and killed. Such a selection, being applied equally to the cold-room and warm-room sections, can have had no influence in determining the differences to be considered later.

After some months' residence in the cold and warm rooms, the mice of the A generation were mated again. This was done simultaneously with the first mating of the B₁ lot. It was believed that such an experiment would furnish a test of the question whether the effects of the temperature conditions would be cumulative from generation to generation. The resulting offspring would, in each case, be reared in the same room as that in which their parents had been kept. The later offspring of the A lot would serve as a control, for comparison, with these offspring of the B₁ lot. The first would be modified by environmental agencies only (assuming that the treatment of their parents during adult life counted for nothing). The second, on the other hand, might give evidence of a heritage from their parents, in addition to these purely somatic modifications.

The resulting young (the B₂ and C₁ lots) were not reared in sufficient numbers to test this point. This was due to the generally high mortality, both prenatal and post-natal, of all these young, aggravated by the fact that during this period of the year, many of the cold-room broods succumbed to the low temperature of the air. Many of the young, especially those of warm-room parentage, were puny and ill-developed, being, in many cases, very scantily clad with hair. Some of these animals remained pink and nearly hairless, even after their eyes were open and they had begun to wander forth from their nests. Others had curious hairless patches on an otherwise well-clad body. In the case of one young mouse, there was a pink stripe on each side of the face, extending through the region where the vibrissae should have been. In some instances, at least, these bizarre effects were not permanent, the hair appearing during later stages of growth. These con-

ditions, shown by the second generation exposed to high temperature, suggest some of Przibram's results above cited (p. 333). I was, myself, disposed at first to attribute them to the cumulative action of heat during two generations. But the force of this conclusion is shaken by the fact that similar individuals—though fewer in number and less striking in appearance—were found among the B₂ lot (i.e., the offspring of A parents) and even in the cold-room section of the C₁ lot. Most of these thin-haired mice were undersized and appeared to be poorly nourished, so that the condition of their pelage was not unlikely the result of mal-nutrition and not to any specific effect of heat.²⁴

None of the mice were mated again until after the discontinuance of the temperature conditions (April 4), when they were transferred to a common room before being paired. The resulting lots (B₃ and C₂) were born at about the same time, and were kept together under identical conditions up to the time of being killed and measured (50 days). The C₂ mice were the offspring of parents which had been modified through temperature conditions; the B₃ ones were the offspring of parents which had been exposed to the same conditions, throughout the same period, but which, having been nearly or quite adult at the outset, underwent little, if any, modification. A comparison between these two lots of young was therefore believed to be highly desirable.

The A mice were not paired again, but the B₁ lot were mated for a third and fourth time. The last of these occasions was about four months after the discontinuance of the temperature conditions.

I have already referred to the fact that both the prenatal and post-natal mortality of certain lots was very high. As an instance of this, I may cite the case of the C₂ lot, in which the total mortality, up to the age of 50 days, was about 60 per cent for the offspring of the cold-room lot, and about 80 per

²⁴ I have, at other times, observed 'runts' which were scantily clad with hair, under circumstances where temperature could have played no part as a cause.

cent for those of warm-room parentage. Indeed, more than half the broods consisted entirely either of still-born young, or of animals which died very early in life. After the first week or two, the mortality was very much lower. But experience makes me confident that by no means all of those which survived to the age of 50 days would have lived to maturity. Indeed, some of these 50-day mice were of such a feeble constitution that they died from exposure, after having been removed from their nests and placed, for a few hours, pending measurement, in open cages, at a temperature by no means low.

In view of this high mortality in the F_1 generation, certain critics may be disposed to rule out of consideration any phenomena exhibited by these mice as merely 'pathological.' I may say, in reply, that this objection does not apply to my 1909 series, in which the mortality was not particularly high. And it is needless to point out that phenomena which are, in reality, pathological may none the less have a high importance for the study of heredity.

To what degree this disastrous mortality resulted from the conditions of my experiment, and to what degree it resulted from an unhealthy condition of my original stock, I am unable to state. It is of significance in this connection that in all three of the F_1 lots to be considered below (' C_2 ,' ' C_3 ' and ' C_4 '), as well as the 1909 lot, already reported on, the mortality was considerably higher among the warm-room descendants. On the other hand, the first offspring of the A generation of mice, born within a few weeks after their arrival at the laboratory, and before they were subjected to any abnormal temperature conditions, likewise exhibited a high death-rate.

It may readily be understood, therefore, why the numbers comprised in my series of mice are in most cases insufficiently great, and why various important experiments were not carried out.

The results of a statistical analysis of these various series of mice will be considered in the next section.

STATISTICAL REVIEW OF THE MEASUREMENTS

1. *The A generation*

Although the weight and tail length of the entire A lot were determined at the commencement of the experiment, I do not regard it as relevant to give these figures here. In table 15 are shown the averages of these first measurements, for those members of the A lot which became the parents and grandparents of mice belonging to our various experimental series. These figures will be considered later. In table 3 are presented mean figures, based upon the final measurements of the surviving A mice (including both parents and non-parents) at

TABLE 3

A generation mice; animals killed and measured May and June, 1911

1. *Gross averages*

		NO.	WEIGHT		BODY LENGTH	TAIL	FOOT	EAR
			gm.	mm.	mm.	mm.	mm.	
Cold-room section.....	Males	25	26.36	95.90	85.58	17.909	14.650	
	Females	60	24.66	97.77	90.16	17.771	14.745	
	Both sexes	85	25.16	97.22	88.81	17.810	14.719	
Warm-room section.....	Males	20	25.51	95.30	87.13	17.642	14.300	
	Females	58	23.83	96.91	90.05	17.633	14.771	
	Both sexes	78	24.26	96.50	89.31	17.636	14.659	
Entire A lot..	Males	Mean	25.98	95.63	86.25	17.785	14.492	
		Stand. dev.	±0.29	±0.32	±0.52	±0.079	±0.060	
	Fe-males	Mean	24.25	97.35	90.11	17.703	14.758	
		Stand. dev.	±0.21	±0.21	±0.28	±0.034	±0.035	
		Both sexes	Mean	3.33	3.37	4.54	0.545	0.565
		Mean	24.73	96.88	89.05	17.725	14.690	

2. *Mean differences ($\sigma - \varphi$)*

(Animals grouped according to body length)

WEIGHT	TAIL	FOOT	EAR
+3.04	-2.37	+0.231	-0.246

the close of the experiments.²⁵ With the exception of some additional males, received early in January, these mice had been kept from the middle of November until April 4 in the two experimental rooms having contrasted temperatures. Since the animals were nearly adult at the commencement of the experiment, little or no modification in respect to the measured parts could be expected. And we find, indeed, that the females, which were older, when received, than the males, display no significant differences between the warm-room and cold-room sections, even though the animals of both of these are known to have grown somewhat during this period of treatment.²⁶ The males, on the other hand, which were less mature than the females, at the beginning of the experiment, exhibit a slight difference, as regards tail length, and a consideration of the first measurements (not here given), shows that this member has actually elongated somewhat more in the warm-room lot than in the cold. This difference is slight, however, in comparison with the changes effected in animals which were subjected from birth to the temperature conditions.

A comparison of males and females, according to the method of size-groups reveals not inconsiderable differences of tail and foot lengths between the two sexes, the former member being longer in the females, the latter in the male.²⁷ The males are likewise considerably heavier, when animals of the same size are compared. The subject of sexual differences will be considered below.

2. *The B₁ lot*

These mice were the ones which were directly modified by temperature conditions acting, in most cases, from the time of birth. They were the parents of the ones in which the question of the reappearance (transmission?) of these modifications

²⁵ The survivors comprised only 60 per cent of the original stock, since there were many deaths before the time of the final measurements.

²⁶ Perhaps a comparison by size-groups might bring to light small, constant differences, but in any case these would be trivial compared with those shown by the B₁ lot.

²⁷ Graphs, not here reproduced, show that these differences were pretty constant, at least in this series.

was investigated. This lot were all subjected to certain measurements at the age of 50 days. For this purpose the males were anesthetized, so that for these it is possible to give foot and ear length, as well as tail length and weight. For the females, which were not anesthetized, it is possible to give the latter measurements only.

In the first division of table 4 we are enabled to compare the mean condition of these parts in cold-room and warm-room sections. In the males, while the mean weight is nearly the same in both cases, the mean tail length is 16 per cent greater for the warm-room than the cold-room animals; the mean foot length is 3 per cent greater; while the mean ear length is less than 1 per cent greater. Reference to the probable errors shows that, as regards tail and foot length, the differences are of undoubted statistical significance. For ear length this cannot be said. Turning to the females, the mean weight is over 7 per cent greater for the warm-room than for the cold-room ones, while the mean tail length differs by an amount almost identical with that shown by the males, i.e., about 16 per cent. Both of these differences, particularly that for tail length, are significant ones.

It has been pointed out that the absolute weight of the warm-room females was greater than that of the cold-room females. The two lots of males, on the other hand, were of nearly equal weight, though even with these the warm-room lot was slightly heavier.

In the experiments of 1907-1908 and 1908-1909 (previously reported) similar conditions were met with. In both of these cases, the warm-room females were heavier than the cold-room ones, while the two lots of males were nearly equal in weight. Here however, the cold-room males were somewhat heavier than those of the warm-room. These differences, as regards absolute weight, may have some general significance, but their meaning is uncertain. Unfortunately, the relative weight, as shown by comparing individuals of the same body length, cannot here be given, since the latter measurement was not made at this period.

TABLE 4

B₁ mice, measured, living, at the age of 50 days; (the males were anesthetized)

1. Gross averages

		NO.	WEIGHT		TAIL*	FOOT	EAR
				gm.	mm.	mm.	mm.
Cold-room lot...	Males	65	Mean	{ 14.45	61.97	17.079	12.592
				{ ±0.25	±0.41	± 0.045	±0.053
			Stand. dev.	{ 2.93	4.94	0.543	0.629
	Females	77	Mean	{ 12.57	61.74		
				{ ±0.17	±0.40		
			Stand. dev.	{ 2.24	5.19		
Both sexes		142	Mean	{ 13.43	61.85		
Mean differences		(♂—♀)		{ +0.01	-2.18		
Warm-room lot (all).....	Males	101	Mean	{ 14.65	71.67	17.599	12.686
				{ ±0.19	±0.37	±0.040	±0.031
			Stand. dev.	{ 2.88	5.56	0.599	0.455
	Females	79	Mean	{ 13.48	71.28		
				{ ±0.16	±0.37		
			Stand. dev.	{ 2.09	4.89		
Both sexes		180	Mean	{ 14.14	71.50		
Mean differences		(♂—♀)		{ -0.02	-1.39		

2. 'a' and 'b' sections of warm-room lot

		NO.	WEIGHT	TAIL*	FOOT	EAR
Section 'a.' (Placed in warm-room immediately after birth).....	Males	68	14.42	71.09	17.526	12.594
	Females	54	13.13	70.48		
Section 'b.' (First kept for two weeks in cold- room).....	Males	33	15.11	72.88	17.748	12.874
	Females	25	14.26	73.00		

3. Mean differences (warm—cold)

• (Animals grouped according to weight)

	TAIL	FOOT	EAR
Males.....	+8.93	+0.493	+0.027
Females.....	+8.08		

* These tail measurements, being made upon living mice, are not quite comparable with those given in most of the other tables; (see p. 349).

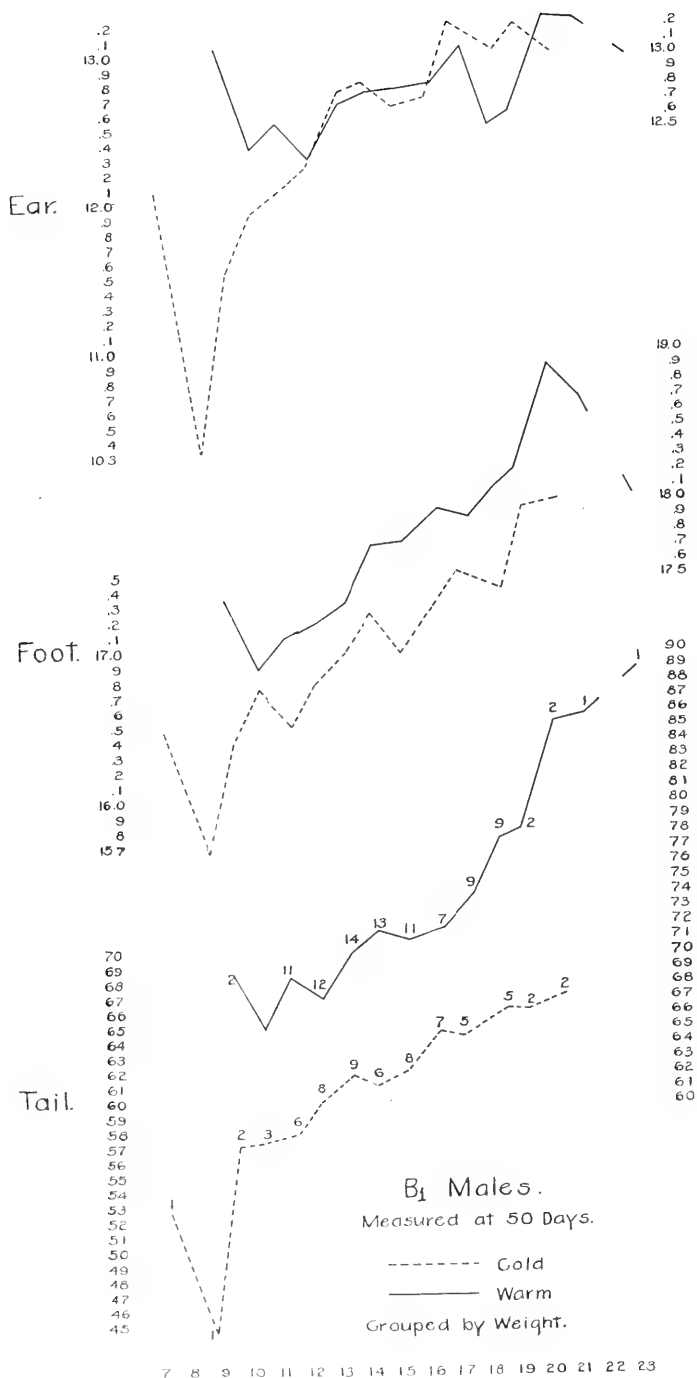


Figure 2

In the second part of table 4 a comparison is made between that section of the warm-room lot which was reared from birth in the warm-room and that section which was first kept for two weeks in the cold-room. Since these two groups of animals differ somewhat in mean size, a comparison of gross averages is not justifiable in the case of the parts measured. Curves (not here reproduced) make it fairly clear, however, that none of the differences shown among them are to be regarded as significant.

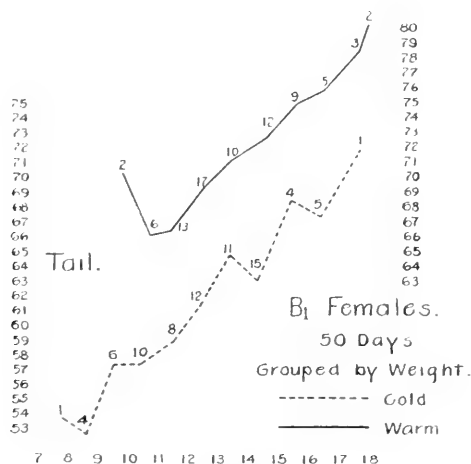


Figure 3

Finally, in section 3 of the table, we have the mean differences between the warm-room and cold-room lots, computed as described above (pp. 345-346).

A graphic comparison between the two contrasted lots of males, as regards tail, foot and ear length, is made in figure 2, while a comparison of the females, as regards tail length, is made in figure 3. It will be seen that the only case in which these differences are not constant and considerable is that of ear length.

In table 5, the results of the measurements of the 'superfluous males' (pp. 354-355), made some ten weeks later than the foregoing, have been presented. It will be seen that the differences in tail and foot lengths have persisted in an unmistakable

manner. It is shown in a later table (26), however, that as regards tail length, the difference diminished during this interval of time, the tails of the cold-room individuals growing more rapidly than those of the warm-room ones. As regards ear length, there is now practically no difference between the gross averages.

In the present table, I have likewise included determinations of hair weight. The quantity of hair is shown to be greater for the cold-room animals, whether absolute values are considered, or relative values, expressing the amount of hair per unit of body surface. Since these differences are in each case about 3 times their probable errors, it is likely that they represent significant differences between these two lots, particularly since the case agrees very well with that of an earlier year (Sumner '09, pp. 129-133).

TABLE 5

B₁ mice; superfluous males, killed and measured at the age of 4 months

1. Gross averages

NO.		WEIGHT	BODY LENGTH	TAIL	FOOT	EAR	HAIR (weight in mg.)	HAIR (ratio weight: square of body length)	
		gm.	mm.	mm.	mm.	mm.			
Cold-room lot...	32	Mean {	21.39	89.77	70.16	17.217	13.789	357.0	0.0444
		±0.27	±0.33	±0.44	±0.056	±0.053	±6.4	±0.0007	
		Stand. dev.	2.28	2.79	3.70	0.474	0.442	53.3	0.0056
Warm-room lot...	52	Mean {	21.02	89.41	79.64	17.788	13.786	332.5	0.0415
		±0.27	±0.37	±0.51	±0.058	±0.042	±5.5	±0.0005	
		Stand. dev.	2.84	3.96	5.49	0.622	0.454	57.8	0.0054

2. Mean differences (warm—cold)

	WEIGHT	BODY LENGTH	TAIL	FOOT	EAR	HAIR (weight in mg.)
Grouped by weight...		-0.15	+10.20	+0.696	-0.002	-23.8
Grouped by body length.....	-0.29		+9.69	+0.635	-0.004	-28.9

In the second part of table 5, the mean differences between the warm-room and cold-room sections of this lot of mice have been computed, according to the method here adopted. It will be seen that the differences obtained are nearly the same whether the animals are grouped by weight or by body length. Those for tail, foot and hair are doubtless significant, while that for weight is perhaps so, in view of the relations shown in figure 4. As regards ear length, the trivial difference indicated in the table is not to be regarded as having any statistical importance. Figure 4, just referred to, shows these various relations graphically. It demands no further comment.

The females and the breeding males were killed and measured at the close of the breeding experiments (table 6).²³ Referring to the gross averages, it will be seen at a glance that the differences in tail length between the cold-room and warm-room animals have very considerably diminished, being only $5\frac{1}{2}$ per cent for the males and $7\frac{1}{4}$ per cent for the females, as compared with a difference of about 16 per cent for each sex previously. In the males, the difference in mean foot length has diminished from 3 per cent to about 2 per cent. A more exact analysis of the growth of these parts during this interval, based upon a consideration of the individual animals, will be offered later. The trivial differences in ear length may be disregarded.

A consideration of the differences in hair weight is interesting, even if not wholly intelligible. The hair of the females only was prepared and weighed, since some of the males were afflicted with a skin disease which diminished the density of the hair on certain parts of the body. These females of the two contrasted lots, containing about fifty animals each, showed a mean difference in favor of the *warm-room* animals of about 29 milligrams, when the gross averages are compared, and this difference is about $3\frac{1}{2}$ times its probable error. Since the animals differed somewhat in both weight and body length (the warm-room lot being the larger) a fairer comparison is effected

²³ Since nearly a third of the animals had died in the meantime, these two groups are not entirely comparable with the corresponding ones some months earlier.

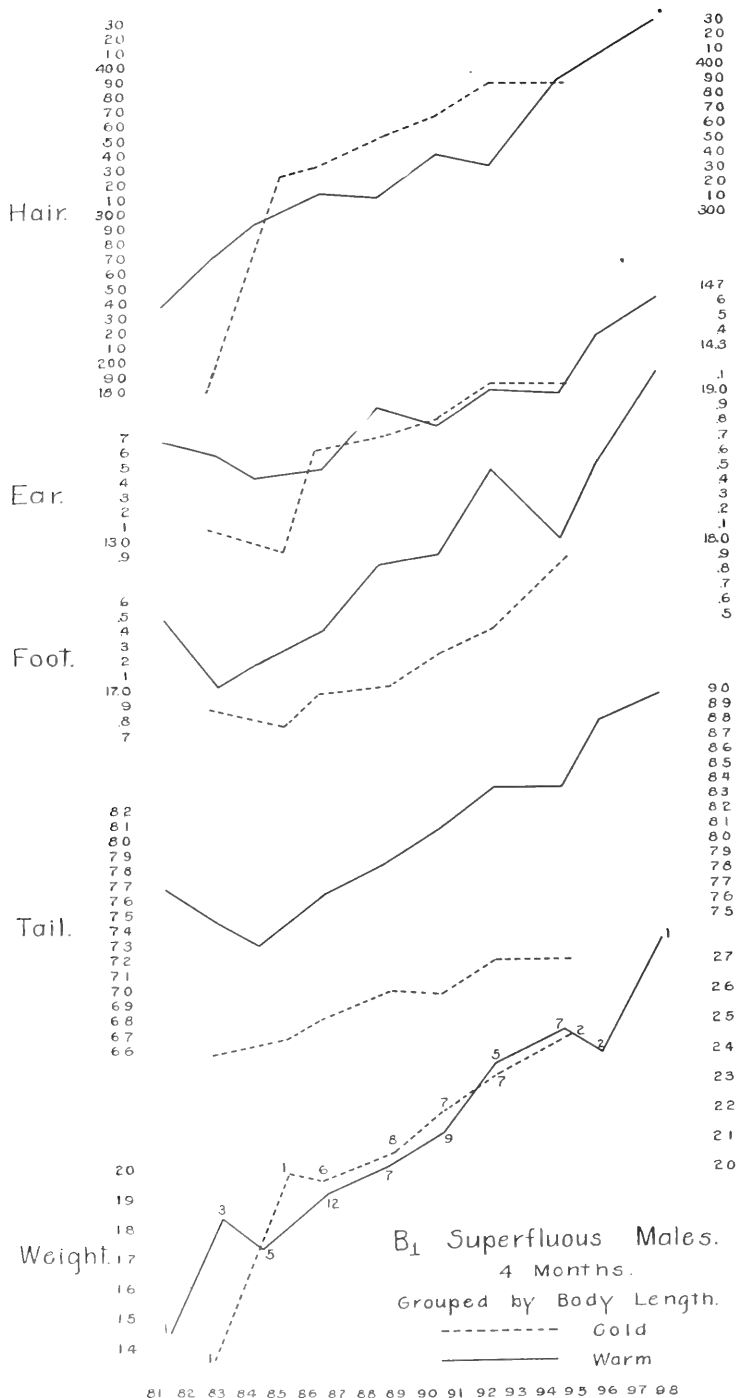


Figure 4

TABLE 6

*B₁ mice; females and breeding males, killed and measured August and September 1911, at the age of 9 to 10 months**

1. Gross averages

		NO.			WEIGHT		BODY LENGTH		TAIL ABSOLUTE		TAIL RELATIVE	FOOT	EAR	HAIR (weight in mg.)
					gm.	mm.	mm.	per cent	mm.	per cent				
Cold-room lot...	Males	24	Mean		22.54	94.87	80.35	84.62	17.393	14.194				
	Fem.	53	Mean		21.78	94.88	79.74	84.07	17.154	14.238				305.8
			Stand. dev.		±0.31	±0.34	±0.43	±0.30	±0.049	±0.048				±4.8
					3.32	3.71	4.63	3.24	0.528	0.498				50.0
	Both sexes	77	Mean		22.02	94.88	79.93		17.227	14.227				
Warm-room lot...	Males	29	Mean		24.27	95.15	84.78	89.14	17.748	14.298				
	Fem.	56	Mean		23.20	96.11	85.47	88.93	17.707	14.265				334.6
			Stand. dev.		±0.27	±0.31	±0.40	±0.36	±0.048	±0.035				±6.8
					2.93	3.42	4.49	4.01	0.535	0.388				72.9
	Both sexes	85	Mean		23.57	95.78	85.23		17.720	14.276				

2. Mean differences (warm—cold; females only)

	WEIGHT	BODY LENGTH	TAIL ABSOLUTE	FOOT	EAR	HAIR (weight in mg.)
Grouped by weight.....		-0.34	+5.09	+0.457	-0.003	+ 8.6
Grouped by body length..	+0.74		+5.27	+0.475	-0.001-	+24.7

* Exceptions are a few animals, particularly males, which died 1 to 3 months earlier, but whose measurements are nevertheless included here; also a few females which, having young, were not killed until October.

by the method of size-groups. It will be seen that the difference is nearly 25 milligrams, when the animals are grouped according to body length and 8.6 milligrams when they are grouped according to weight.²⁹ Unfortunately a comparison

²⁹ Possibly some part of the difference between these two figures is due to the fact that, with both methods of grouping, certain unpaired groups, at one or the other end of the series, cannot be included in the computation of differences. Thus the comparison, in the two cases, is not made between exactly the same two lots of individuals. Much of the difference here, however, must be due to the fact, shown in the table, that, when animals of equal length are compared, the warm-room individuals are in general somewhat heavier, while, in comparing animals of equal weight, the warm-room ones are slightly shorter.

with the earlier condition of the females is impossible for this character, but we have no reason for supposing that the behavior of the two sexes, as regards growth of hair, is different. When we compare the final condition of these females, with that earlier shown by the 'superfluous males,' it does not seem improbable that the original differences in the quantity of hair, produced by temperature differences, were, for some reason, reversed after these latter conditions had been discontinued.

Another matter of some interest is the greater absolute size of the warm-room animals, of both sexes, as compared with those reared in the cold-room. In the second part of table 6, there is likewise indicated a considerably greater *relative* weight for the warm-room females in comparison with the cold-room ones (i.e., when animals of equal size are compared). The males, being much less numerous than the females, were not compared by size-groups for most of the characters. I have now done this for weight, however, with the result that the mean difference is 1.77 gram, in favor of the warm-room lot. Moreover, this difference has the same sign in each of the six size-groups compared. Thus, for this particular lot, at this time of life, warm-room animals of a given body length are heavier than cold-room animals, and this is true of both sexes. Unfortunately, I have but few other data indicating the relative weight of mice of equal size which have been reared at low and high temperatures. As has already been stated, the other ('superfluous') males of this lot, which were killed at the age of four months, showed a reverse difference, though of smaller amount. In my experiments of earlier years (1907 and 1908), no comparison was made by size-groups, but, so far as may be judged from the gross averages, the conditions were contradictory in different lots. But whether or not there be shown any general effect of temperature upon the relative body weight of mice, it must be borne in mind that in the breeding animals of both sexes among the B₁ lot (i.e., the parents of the various C broods), the warm-room animals were heavier relatively as well as absolutely.

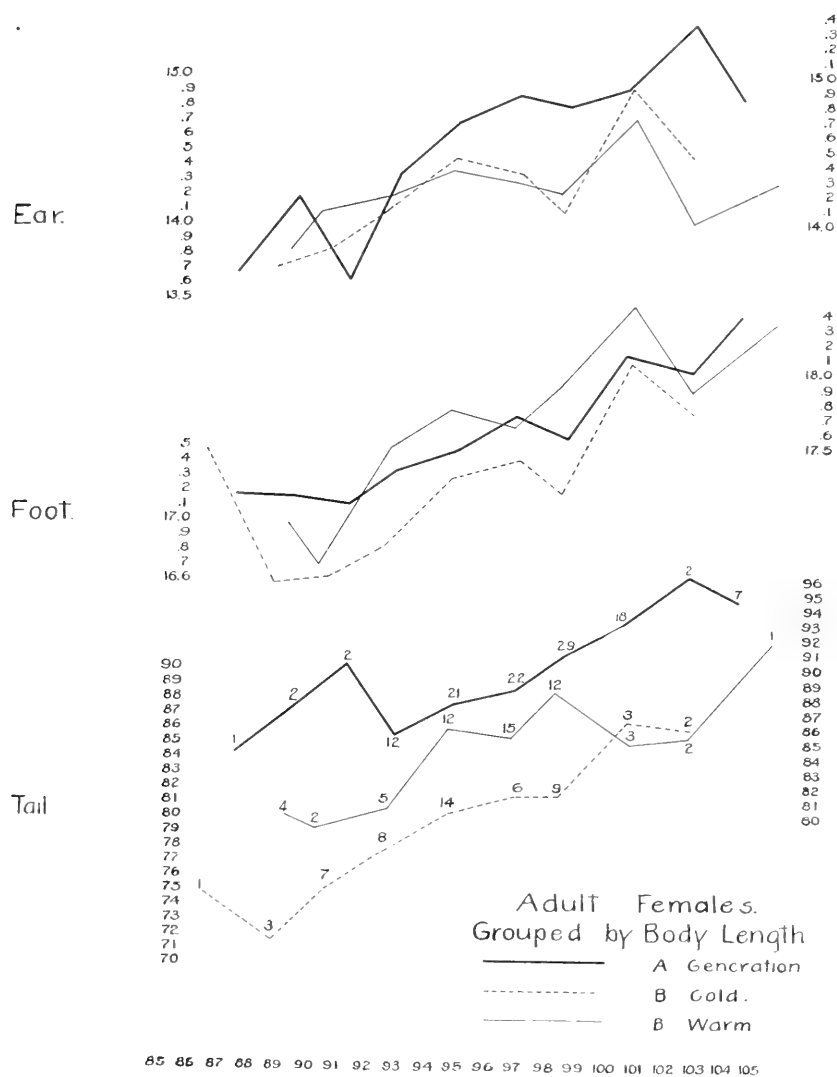


Figure 5

Figures 5 and 6 permit of a comparison between the warm-room and cold-room sections of the B₁ females, figure 5 likewise including curves for the females of the A generation (both sections combined). These figures agree with the table in

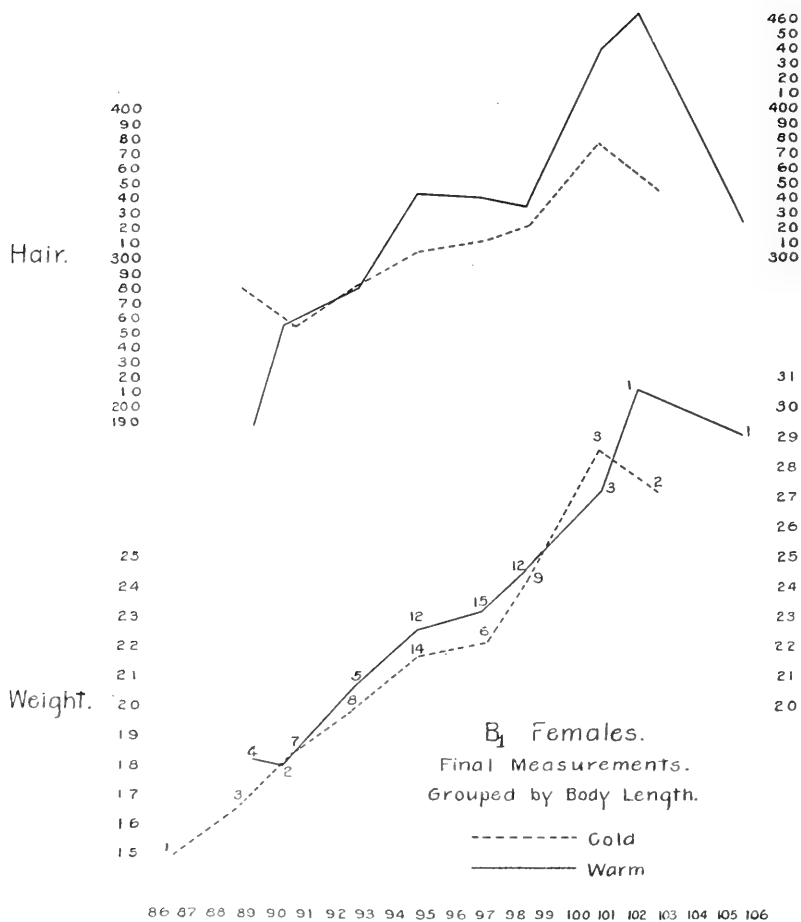


Figure 6

showing for the B₁ lot, that body weight, quantity of hair, and the length of tail and foot are unmistakably greater in the warm-room section, while ear length presents no certain differences. A comparison between the A and B₁ females is likewise instructive. It is rather surprising to find the A lot possessed of decidedly longer tails, both relatively and absolutely, than even the warm-room section of the B₁ lot, while in ear length the former likewise considerably exceeds the latter.

As regards foot-length, the A animals are intermediate between the two B_1 series, though nearer, on the whole, to the warm-room than the cold-room condition. Of course the mice of the A generation, being the parents of the B animals, were considerable older than the latter at the time of these measurements, but even these last were about ten months old and therefore fully mature. Whether or not, at this time of life, the appendages still continue to grow has not been ascertained with certainty, but it seems highly improbable.

3. The B_3 lot

As already stated, the B_2 lot (second offspring of the A mice) were too much reduced in numbers to be of any service for measurements. A fair number of surviving young resulted, however, from the third mating of the A animals, at least as regards the cold-room section. It must be borne in mind that the parent lot had been subjected, throughout some months, to the same extremes of temperature as their earlier offspring (B_1), but that, being nearly or quite mature at the outset, they were little, if at all, modified by the treatment. The B_3 young were all reared together in a common room.

Table 7 and figures 7 and 8 present the results from the measurement of the B_3 lot, at the age of 50 days. The number of those born of warm-room parents was so small (50) that no very satisfactory comparison can be made with those of cold-room parentage. The second section of the table shows a series of differences having, with a single exception, the same sign, indicating slightly larger measurements for mice of cold-room parentage. And the curves likewise show fairly constant differences in certain cases, sometimes in favor of one set, sometimes of the other. But in most of the pairs of curves, these latter cross one another so capriciously that little confidence can be placed in the differences as a whole. In this respect they are in contrast with some of those to be discussed later.

The chief interest of the B_3 mice lies, however, in the comparison, to be made presently, between these and another

lot of young, reared under identical conditions, but born of parents which had been considerably modified by temperature differences acting from birth.

TABLE 7
B₃ mice; killed and measured at the age of 50 days

1. Gross averages

			NO.		WEIGHT	BODY LENGTH	TAIL ABSOLUTE	TAIL RELATIVE	FOOT	EAR
					gm.	mm.	mm.	per cent	mm.	mm.
Of cold-room parentage..	Males	61	Mean	{	12.12	74.31	69.26	93.55	17.815	12.979
					±0.28	±0.50	±0.51	±0.39	±0.055	±0.045
			Stand. dev.	3.19	5.79	5.67	4.28	0.632	0.516	
	Females	46	Mean	{	11.54	73.38	69.44	94.55	17.592	12.822
					±0.24	±0.55	±0.50	±0.49	±0.048	±0.047
			Stand. dev.	2.39	5.49	4.90	4.82	0.484	0.473	
Both sexes	107	Mean		11.87	73.91	69.34		17.718	12.912	
Of warm-room parentage..	Males	20	Mean	{	10.96	71.70	67.92	95.05	17.502	12.612
					±0.34	±0.78	±0.82	±0.53	±0.105	±0.093
			Stand. dev.	2.23	5.19	5.28	3.44	0.694	0.617	
	Females	30	Mean	{	11.01	72.53	69.03	95.33	17.363	12.662
					±0.31	±0.65	±0.59	±0.62	±0.076	±0.068
			Stand. dev.	2.55	5.26	4.83	5.07	0.615	0.552	
Both sexes	50	Mean		10.99	72.20	68.60		17.419	12.642	

2. Mean differences (warm—cold; grouped according to body length)

	WEIGHT	TAIL ABSOLUTE	FOOT	EAR
Males.....	-0.14	+1.12	-0.062	-0.211
Females.....	-0.11	-0.44	-0.178	-0.126

3. Mean differences (♂—♀; grouped according to body length)

	WEIGHT	TAIL ABSOLUTE	FOOT	EAR
Of cold-room parentage.....	+0.13	-0.45	+0.125	+0.105
Of warm-room parentage.....	+0.17	+0.35	+0.415	+0.092

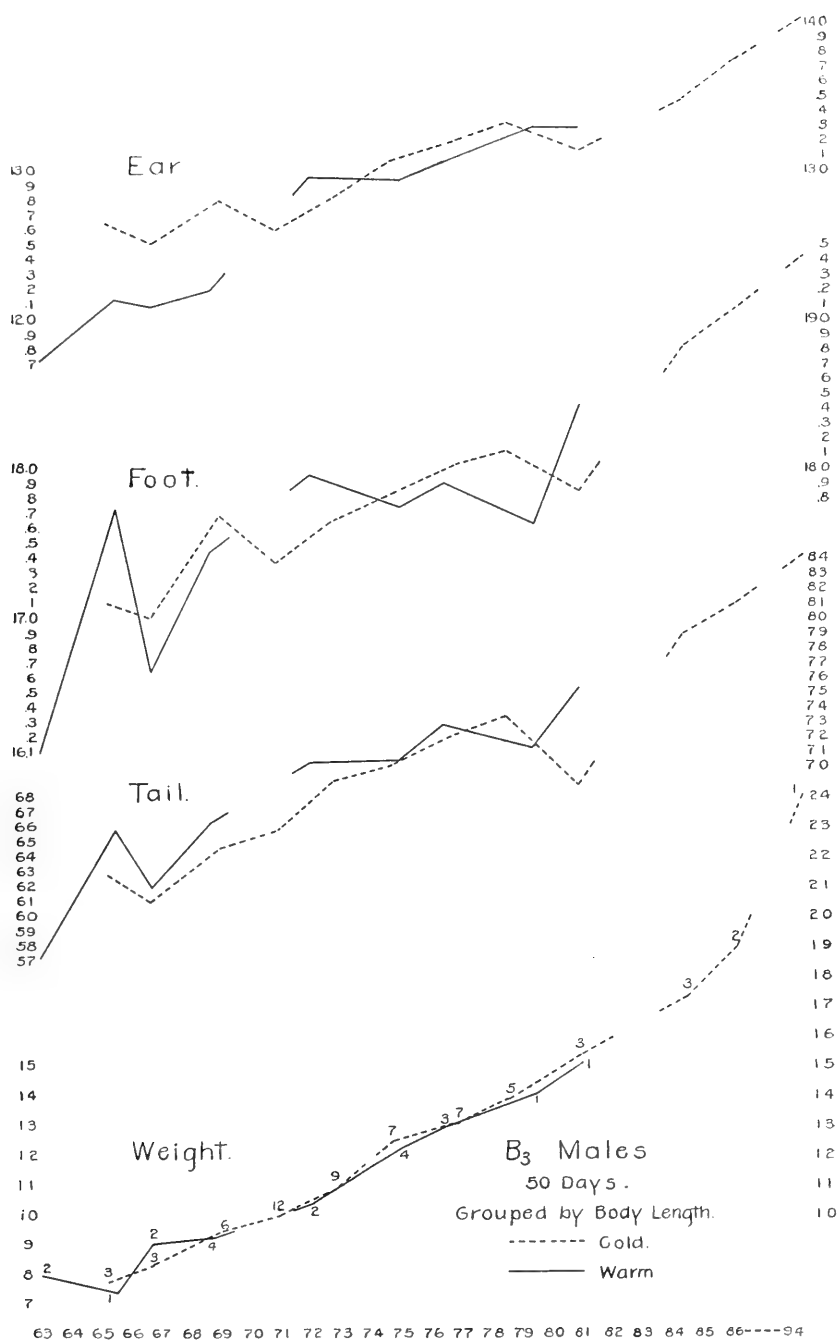
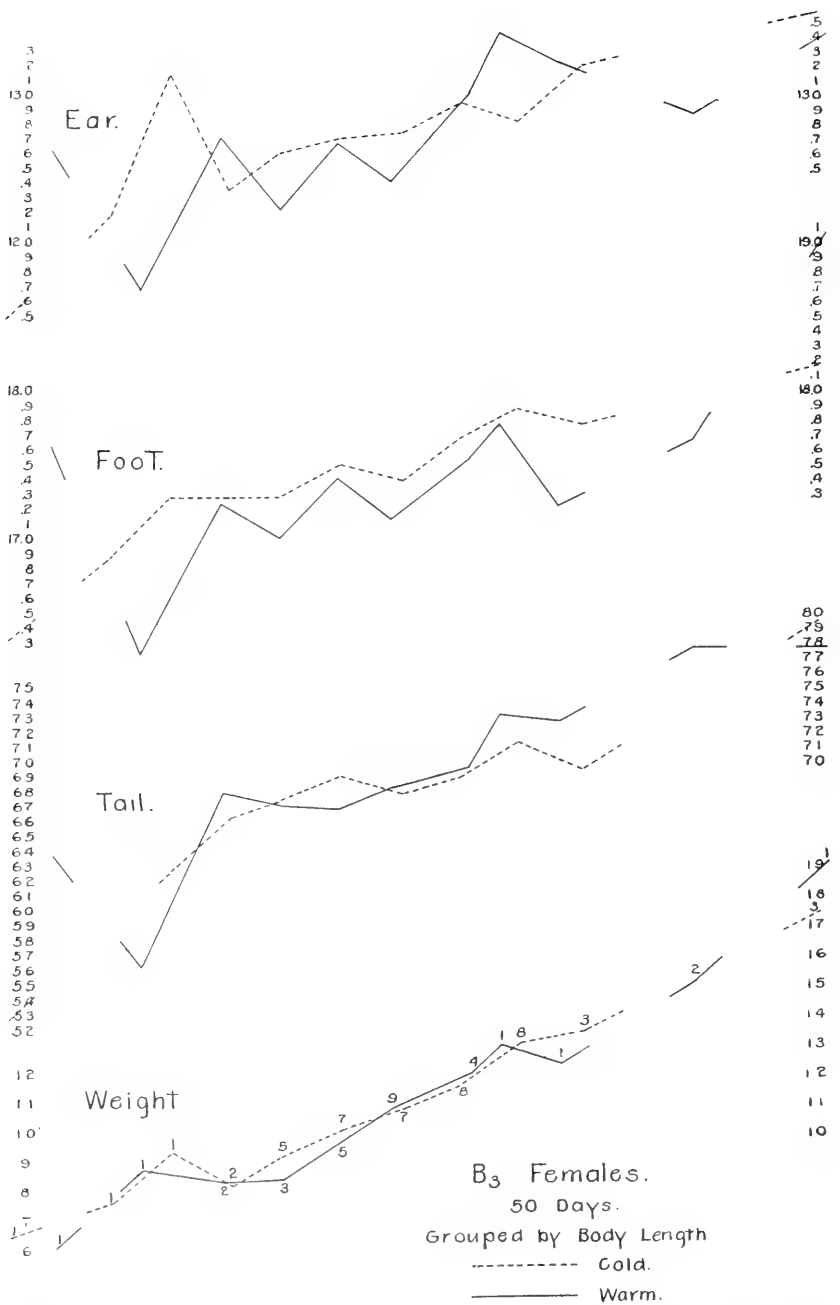


Figure 7



53 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87

Figure 8

4. The C₂ lot

These were the offspring of modified parents (B₁), though themselves conceived and reared in a common room. From an inspection of the gross averages in table 8, it will be seen that although those of warm-room parentage, males and females, averaged slightly less in body length than those of cold-room parentage, they had on the average somewhat longer tails, feet and ears than the latter. And even these differences between the gross averages, based on absolute magnitudes, are, for tail and foot length, $1\frac{1}{2}$ to 3 times their probable errors. As regards *relative* tail length the difference between the two lots is nearly 6 times its probable error for the males and $4\frac{1}{2}$ times for the females. Both of these differences may therefore be regarded as of practically certain significance, statistically at least. What they mean biologically, is of course another matter.

In the second subdivision of table 8, giving the mean differences between the two contrasted lots, it is seen at once that for tail, foot and ear length, the difference is always in favor of the warm-room descendants, whether the animals are grouped by weight or by body length. When the latter method of grouping is employed, it becomes evident that, for individuals of the same length, the weight is also somewhat greater among the warm-room than the cold-room descendants. It has been shown above (pp. 368-369) that this relation likewise held true for the contrasted sections of the parent (B₁) lot.

It will be shown later that the size of tail, foot and ear are strongly correlated with body weight, and it might therefore be suggested that the greater length of these members in the warm-room descendants resulted from the latter being somewhat heavier mice. When we group our animals according to weight, however, we observe the mean differences to be nearly as great as before, despite the fact that for mice of equal weight, the warm-room descendants are of somewhat *inferior* body length.

Figures 9 and 10 show these relations for the males and females respectively. For both sexes, the 'warm' curve lies,

TABLE 8

C₂ mice; killed and measured at the age of 50 days

1. Gross averages

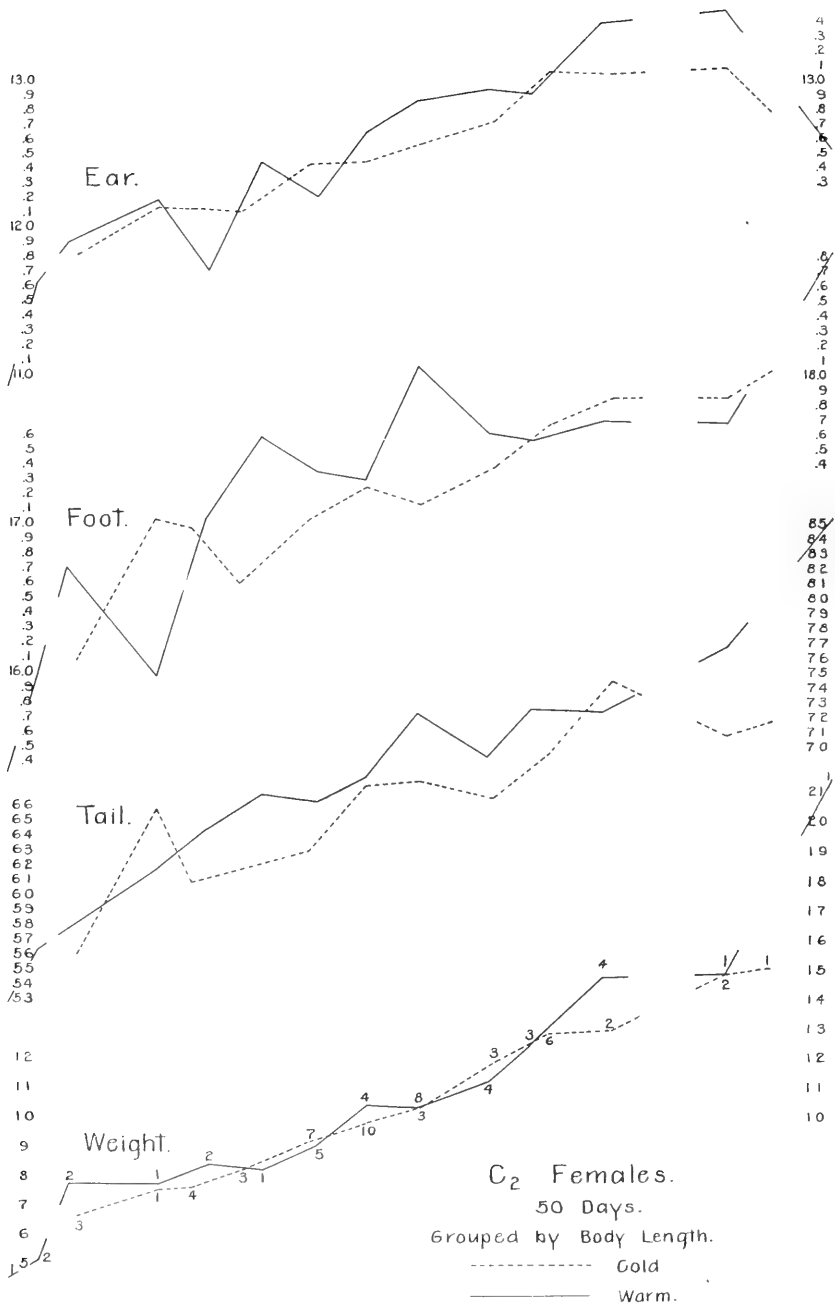
		NO.		WEIGHT	BODY LENGTH	TAIL ABSOLUTE	TAIL RELATIVE	FOOT	EAR
				gm.	mm.	mm.	per cent	mm.	mm.
Of cold-room parentage..	Males	44	Mean	10.58	71.91	66.02	91.86	17.419	12.551
			Stand. dev.	±0.21	±0.47	±0.60	±0.58	±0.069	±0.049
				2.02	4.58	5.72	5.55	0.679	0.478
	Females	50	Mean	10.40	71.16	66.28	93.10	17.202	12.530
			Stand. dev.	±0.21	±0.51	±0.49	±0.49	±0.056	±0.044
	Both sexes	94	Mean	10.49	71.51	66.16		17.304	12.540
Of warm-room parentage..	Males	31	Mean	10.35	70.60	67.87	96.10	17.716	12.585
			Stand. dev.	±0.30	±0.67	±0.52	±0.48	±0.079	±0.060
				2.45	5.56	4.24	3.93	0.656	0.486
	Females	34	Mean	10.72	70.78	67.97	96.39	17.337	12.553
			Stand. dev.	±0.39	±0.90	±0.81	±0.55	±0.091	±0.081
	Both sexes	65	Mean	10.54	70.69	67.92		17.518	12.568

2. Mean differences (warm—cold)

		WEIGHT	BODY LENGTH	TAIL ABSOLUTE	FOOT	EAR
Grouped according to weight.....	Males		-0.95	+2.22	+0.344	+0.048
	Females		-0.51	+2.06	+0.230	+0.033
Grouped according to body length.....	Males	+0.14		+2.48	+0.390	+0.039
	Females	+0.21		+2.39	+0.261	+0.061

3. Mean differences (♂—♀; grouped according to body length)

	WEIGHT	TAIL	FOOT	EAR
Cold.....	+0.11	-1.19	+0.224	+0.013
Warm.....	-0.13	-0.30	+0.243	-0.080



53-56 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85-92

Figure 10

in every instance, above the 'cold' one, throughout the greater part of its course, and in some cases the relative positions of the two remain pretty constant throughout their entire extent. This relation is less obvious for weight and ear length than for the length of the tail and foot. In interpreting these figures, it must be borne in mind, as already remarked, that the more central portions of the various curves are much more significant than the terminal portions, since the former are based upon much larger numbers than the latter.

TABLE 9

Mean differences (B_3-C_2 ; grouped according to body length)

		WEIGHT	TAIL ABSOLUTE	FOOT	EAR
		gm.	mm.	mm.	mm.
Of cold-room parentage.....	Males	+0.35	+1.82	+0.131	+0.220
	Females	+0.26	+1.73	+0.263	+0.140
Of warm-room parentage.....	Males	+0.25	-0.92	-0.263	+0.022
	Females	-0.09	-1.21	-0.323	-0.091

In table 9 a comparison has been made, by the method of size-groups, between the C_2 mice, which had modified parents, and the B_3 mice, whose parents, being mature at the commencement of the experiment, were little if at all modified by exposure to the temperature differences. The latter may, in a sense, be regarded as furnishing a 'control' for the former, since the two lots were reared together in a common room. It will be seen at a glance that, as regards the mice of cold-room parentage, all of the differences are positive, i.e., the B_3 animals are heavier and have longer appendages. This might reasonably have been expected, on the supposition that the C_2 animals were modified in the same direction as their parents. For the warm-room animals, the figures are, with two exceptions, negative, i.e., the quantities represented were *greater* in the C_2 lot. This, also, is what might have been predicted on the above assumption. The 'cold' figures are much more significant than the 'warm' ones, however, since the

former are based upon 107 and 94 individuals, respectively, the latter upon 50 and 65 individuals.

Figures 11 and 12 permit of a graphic comparison of the cold-room sections of these lots, for males and females respectively. The relations which have appeared in the tables are here clearly portrayed, and the figures demand no further comment. The curves for the warm-room sections are not here reproduced, since they are based upon such small numbers. They show, on the whole, however, the same relations as are indicated in table 9.

While these comparisons between the B_3 and the C_2 lots are extremely interesting, and while they harmonize very well with the conditions observed in the respective parent lots, we should be guarded in drawing conclusions from this fact. The differences, already referred to (pp. 368-371) between the A and B_1 mice, when fully mature, and those to be considered later, which were exhibited by the various C lots among themselves (pp. 389-395), make it plain that very considerable differences in type may arise between two lots, which are due to wholly unknown causes.

5. *The C_3 lot*

This was the third lot of offspring derived from the modified (B_1) animals. It resulted from the mating of the parents some 2 months after the withdrawal of the temperature differences. These mice were measured living at the age of 50 days and saved for later measurements. Body length is therefore not represented in these earlier figures. It will be seen (table 10) that for tail and foot, the gross averages are in each case *larger* for the cold-room series, but it will likewise be seen that the latter animals are somewhat heavier and, presumably, somewhat longer. When the animals are compared by size-groups, the mean tail length is still found to be less in both sexes, for the warm-room animals, though these negative figures are much smaller than the positive ones shown in table 8. The males of the cold-room set likewise had somewhat longer feet, but this was not true of the females. Finally, for ear length, the

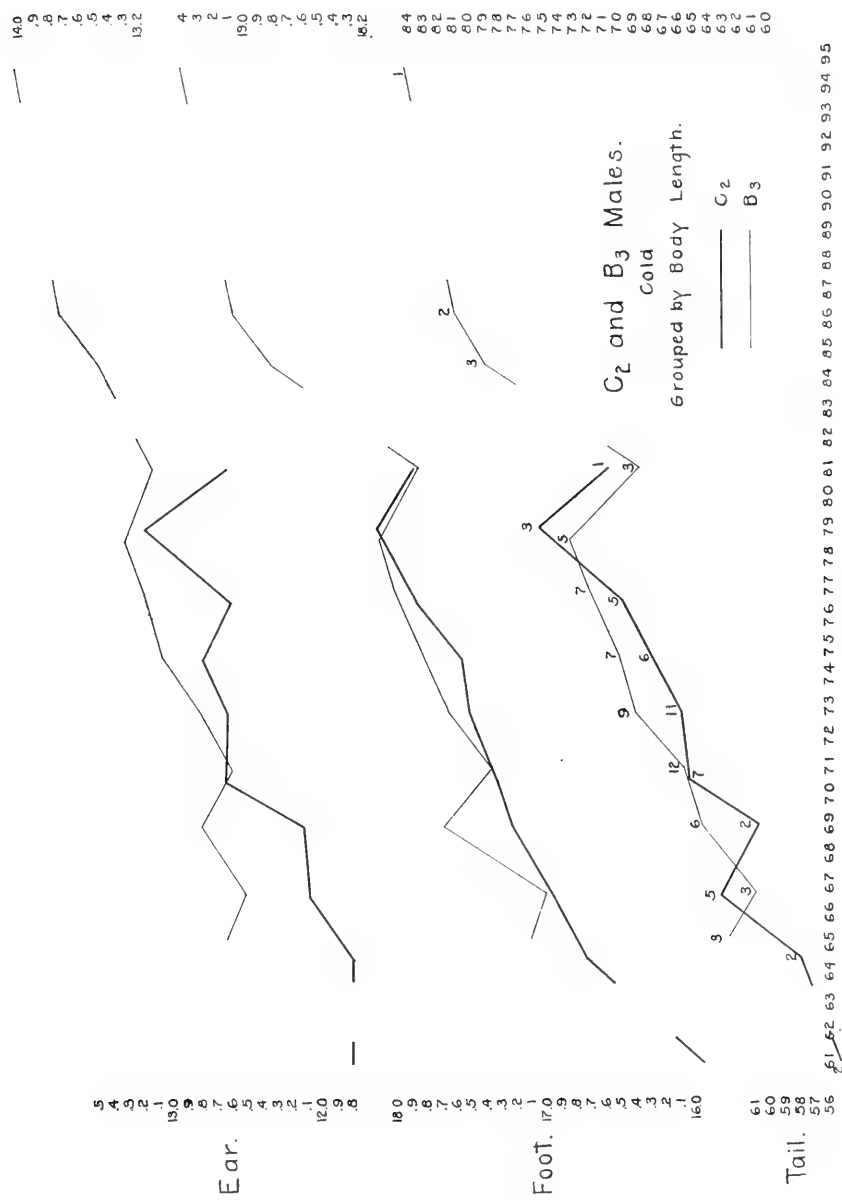


Figure 11

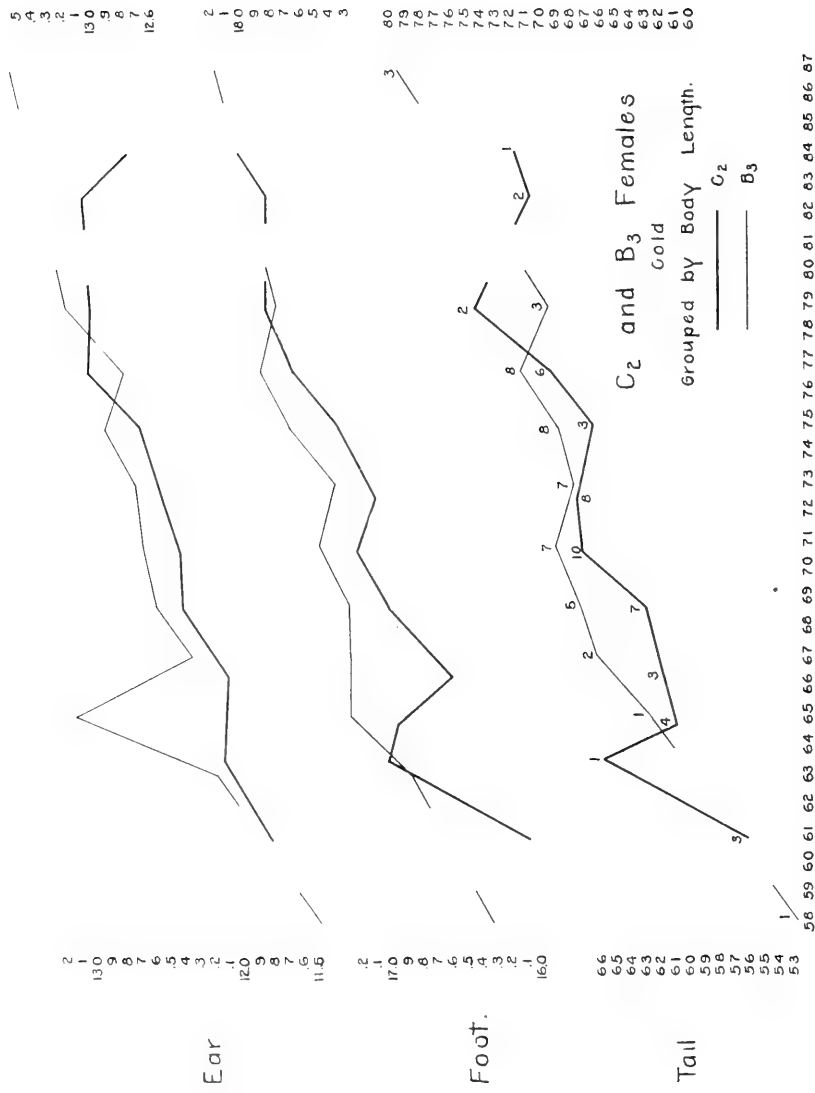


Figure 12

TABLE 10

C₃ mice; measured living at age of 50 days

1. Gross averages

	NO.	WEIGHT		TAIL*	FOOT	EAR
		gm.	mm.	mm.	mm.	mm.
Of cold-room parentage....	Males	56	10.19	65.71	17.227	12.439
	Females	64	10.72	67.73	17.130	12.527
	Both sexes	120	10.47	66.78	17.175	12.486
Of warm-room parentage..	Males	55	9.91	64.47	17.033	12.474
	Females	49	10.42	66.83	17.121	12.610
	Both sexes	104	10.15	65.58	17.074	12.538

2. Mean differences (warm—cold; grouped according to weight)

	TAIL	FOOT	EAR
Males.....	-0.85	-0.140	+0.112
Females.....	-0.29	+0.030	+0.107

* These tail measurements, being made upon living mice, are not quite comparable with those given in most of the other tables; (see p. 340).

differences both bear the same sign as in the C_2 lot. Graphs, not here reproduced, show much crossing and recrossing on the part of the 'cold' and 'warm' curves, indicating a low degree of constancy in these relations.

This lot of mice (or the survivors among them, for a considerable number died) were killed and again measured at the age of three months. The figures are given in table 11. Here the gross averages for tail and foot are seen to be somewhat greater in the cold-room descendants, of both sexes, despite the somewhat smaller mean weight and body length of these. In only one case, however, is this difference as much as equal to its probable error. The gross averages for ear length are in both sexes greater for the warm-room lot. For *relative* tail length (ratio to body), the differences are both in favor of the cold-room animals. They are 1.22 and 1.85 for the males and females respectively, thus being very much less than the reverse differences found in the C_2 lot. Likewise, the statis-

tical certainty of these differences, as judged by their probable errors, is much less than in the case of the earlier lot.

Considering the mean differences based on the method of size-groups, we find that in both sexes those for weight and

TABLE 11
C₃ mice; killed and measured at the age of 3 months

1. Gross averages

		NO.		WEIGHT	BODY LENGTH	TAIL ABSOLUTE	TAIL RELATIVE	FOOT	EAR
				gm.	mm.	mm.	per cent	mm.	mm.
Of cold-room parentage.	Males	42	Mean	18.03	83.93	74.44	88.81	17.511	13.126
			Stand. dev.	±0.33	±0.61	±0.56	±0.44	±0.071	±0.055
				3.22	5.85	5.34	4.25	0.663	0.528
	Females	54	Mean	16.46	82.64	74.72	90.42	17.470	13.322
			Stand. dev.	±0.17	±0.33	±0.44	±0.32	±0.059	±0.045
				1.90	3.55	4.69	3.41	0.624	0.490
Both sexes	96	Mean	17.15	83.20	74.60		17.488	13.236	
		Stand. dev.	±0.32	±0.57	±0.57	±0.37	±0.052	±0.045	
			3.22	5.73	5.78	3.75	0.519	0.457	
Of warm-room parentage.	Males	46	Mean	17.09	83.14	73.70	88.57	17.438	13.538
			Stand. dev.	±0.23	±0.41	±0.55	±0.46	±0.078	±0.050
				2.20	3.91	5.20	4.32	0.708	0.481
	Females	42	Mean	17.97	83.75	73.78		17.476	13.466
			Stand. dev.						

2. Mean differences (warm—cold); grouped according to body length)

	WEIGHT	TAIL ABSOLUTE	FOOT	EAR
Males.....	+0.57	-0.38	-0.022	+0.200
Females.....	+0.30	-1.77	-0.075	+0.227

3. Mean differences (♂—♀); grouped according to body length)

	WEIGHT	TAIL ABSOLUTE	FOOT	EAR
Cold.....	+0.74	-1.50	-0.120	-0.291
Warm.....	+1.08	-0.16	-0.093	-0.238

ear length agree in sign with the corresponding figures in table 8. The figures for tail and foot length are, however, both of opposite sign, though the differences represented are much smaller than in the case of the C_2 lot. Indeed those for the males can hardly be regarded as significant at all.

Curves depicting these relations constitute figures 13 and 14. For weight and ear length, constant differences are shown by both sexes, the 'warm' curve being uppermost. For tail and foot length a reverse relation is shown by the females, and for tail length by the males as well, though this condition is not so evident in the latter case. The males curves for foot length, on the other hand, show no constancy in their relative positions.

In the C_3 lot, therefore, I encountered conditions which were partially at variance with those observed in two previous lots of mice having a similar history. I refer to the C_2 lot, above described, and to that of the 1909 experiment, an account of which has already been published. My first thought was that this exceptional behavior might be due to the fact that the parents of the C_3 mice were mated some time after their removal from the experimental temperature conditions. The effect of the latter might be supposed to have disappeared, as in the case of Przibram's experiments, above discussed (pp. 333-334). But an examination of the C_4 lot, born 2 months later still, quite invalidates this explanation.

6. *The C_4 lot*

These mice were, as already stated, born nearly 5 months after the discontinuance of the temperature differences to which the parents had been subjected. The results of the measurements are given in table 12. An inspection of the gross averages shows that, as in the C_3 lot, both weight and body length were greater for the warm-room animals. On this account, the corresponding absolute differences in tail, foot and ear length, and hair weight would not in themselves be convincing, despite the fact that they are in each case several (2 to 5) times their probable errors. But an examination of the

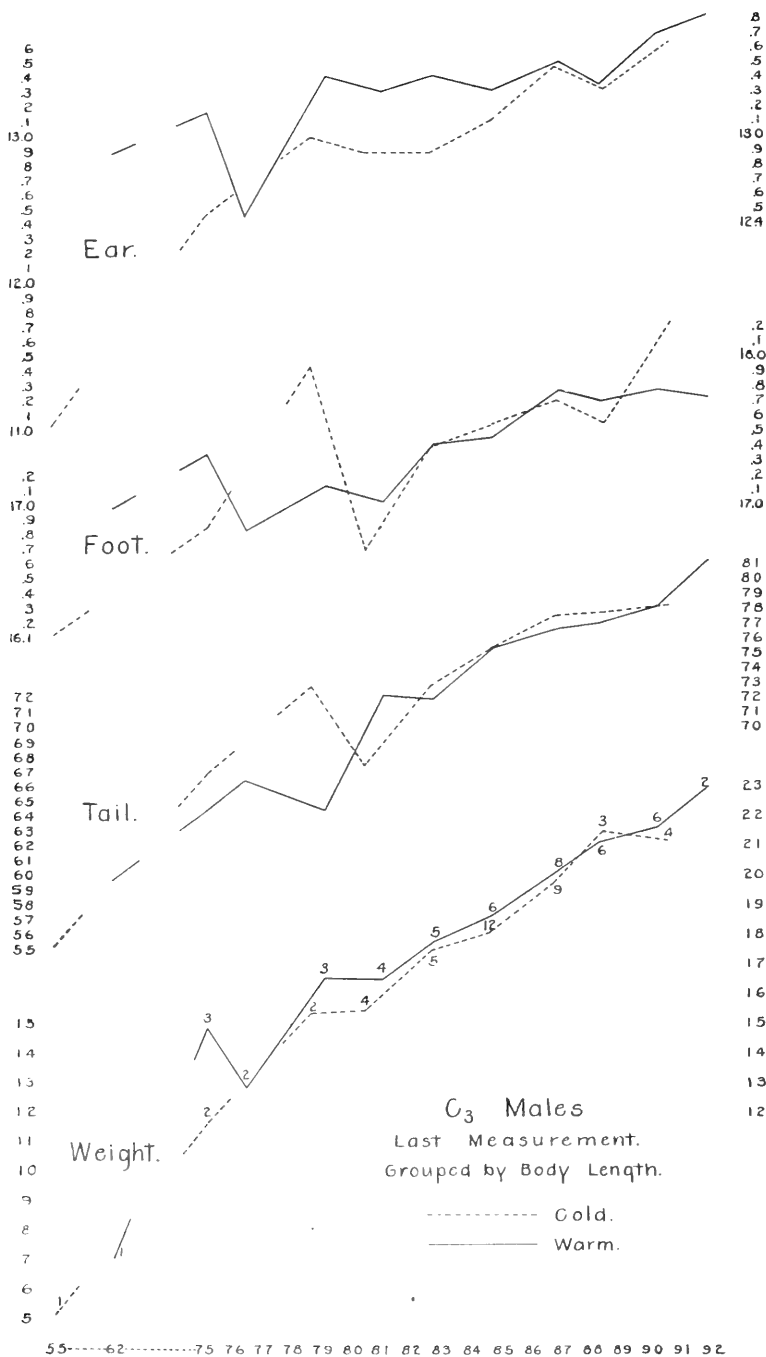


Figure 13

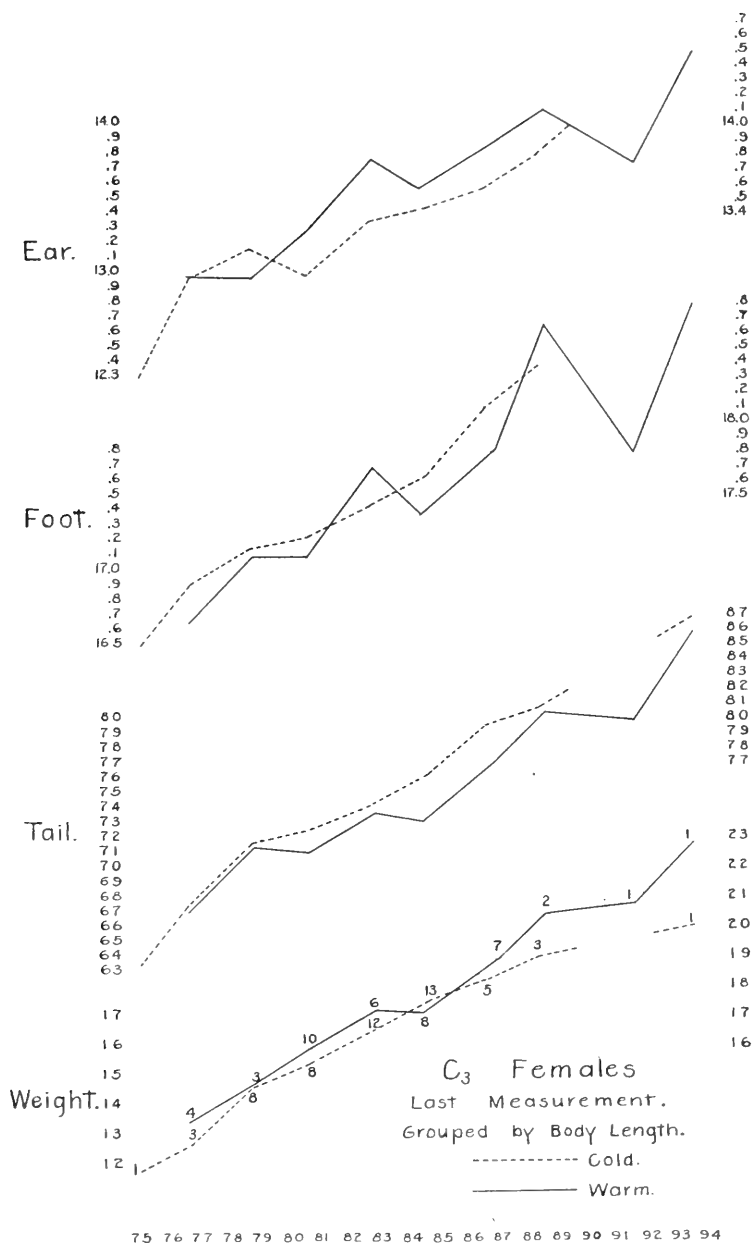


Figure 14

TABLE 12
C₄ mice; killed and measured at the age of 50 days

1. Gross averages

		NO.		WEIGHT	BODY LENGTH	TAIL ABSOLUTE	TAIL RELATIVE	FOOT	EAR	HAIR (weight in mg.)		
					gm.	mm.	per cent	mm.	mm.			
Of cold-room parent-age...	Males	60	Mean	{	9.83	69.82	63.01	89.66	17.302	12.666	196.5	
			Stand. dev.	{	±0.18	±0.45	±0.46	±0.45	±0.051	±0.043	±6.2	
	Fem.	58	Mean	{	9.14	68.11	61.55	90.34	16.989	12.483	166.2	
			Stand. dev.	{	±0.18	±0.46	±0.50	±0.44	±0.056	±0.054	±5.3	
	Both sexes		118	Mean		9.49	68.98	62.27		17.148	12.576	181.8
	Of warm-room parent-age...	Males	52	Mean	{	10.45	70.61	65.57	92.41	17.498	12.879	214.7
Stand. dev.				{	±0.21	±0.49	±0.57	±0.50	±0.065	±0.053	±7.2	
Fem.		41	Mean	{	9.69	68.34	65.19	94.74	17.337	12.877	186.8	
			Stand. dev.	{	±0.21	±0.65	±0.44	±0.53	±0.070	±0.060	±7.0	
Both sexes		93	Mean		10.11	69.61	65.41		17.428	12.878	202.4	

2. Mean differences (warm—cold)

		WEIGHT	BODY LENGTH	TAIL ABSOL- UTE	FOOT	EAR	HAIR
Grouped according to weight.....	{ Males	-0.31	+1.55	+0.042	+0.087	-0.3	
	{ Females	-0.89	+2.55	+0.296	+0.251	+6.2	
Grouped according to body length...	{ Males	+0.25	+2.46	+0.137	+0.142	+11.1	
	{ Females	+0.51	+3.15	+0.429	+0.359	+17.1	

3. Mean differences (σ — φ); grouped according to body length)

	WEIGHT	TAIL	FOOT	EAR	HAIR
Cold.....	+0.05	-0.67	+0.148	+0.014	+6.2
Warm.....	-0.12	-0.69	-0.069	-0.106	+7.4

figures for relative tail length reveals differences which are 4 and 6 times their probable errors, respectively, for the males and females.

Turning to the 'mean differences' in the next section of our table, we find positive figures for tail, foot and ear length, whichever method of grouping be adopted. For hair weight, we find two rather large positive differences, when the animals are grouped according to body length, though these differences are greatly diminished (in one case being of reverse sign, though negligible) when the animals are grouped according to weight. These last facts are not very intelligible, though the general correspondence between these relations and those shown by the female parents in the second part of table 6 is at least suggestive. In respect to pelage, it would seem that these C_4 mice resemble the later condition of their parents, rather than the earlier condition, as shown in table 5.

When animals of equal body length are grouped together, it appears that, as in the C_2 and C_3 lots, those of warm-room parentage are appreciably heavier than those of cold-room parentage. Accordingly, among animals of the same weight, the warm-room descendants average slightly *shorter* than the cold-room descendants, a fact which makes still more significant their somewhat longer appendages.

In figures 15 and 16 some of the relations just discussed are portrayed graphically. It is to be noted that the curves for tail and foot length in the males greatly exaggerate the exceptional cases. It will be seen that the 63-millimeter group of warm-room animals, which is chiefly responsible for the marked dip of the 'warm' curve below the 'cold' one, contains but a single individual. Otherwise, these figures require no comment.

7. *The C_2 , C_3 and C_4 lots compared*

Hitherto, our comparisons have been chiefly between the 'cold' and 'warm' sections of the same lot of animals. But it is also important to compare the corresponding section ('cold' or 'warm') in the different F_1 lots (C_2 , C_3 and C_4). My tables are not adapted to making such a comparison very

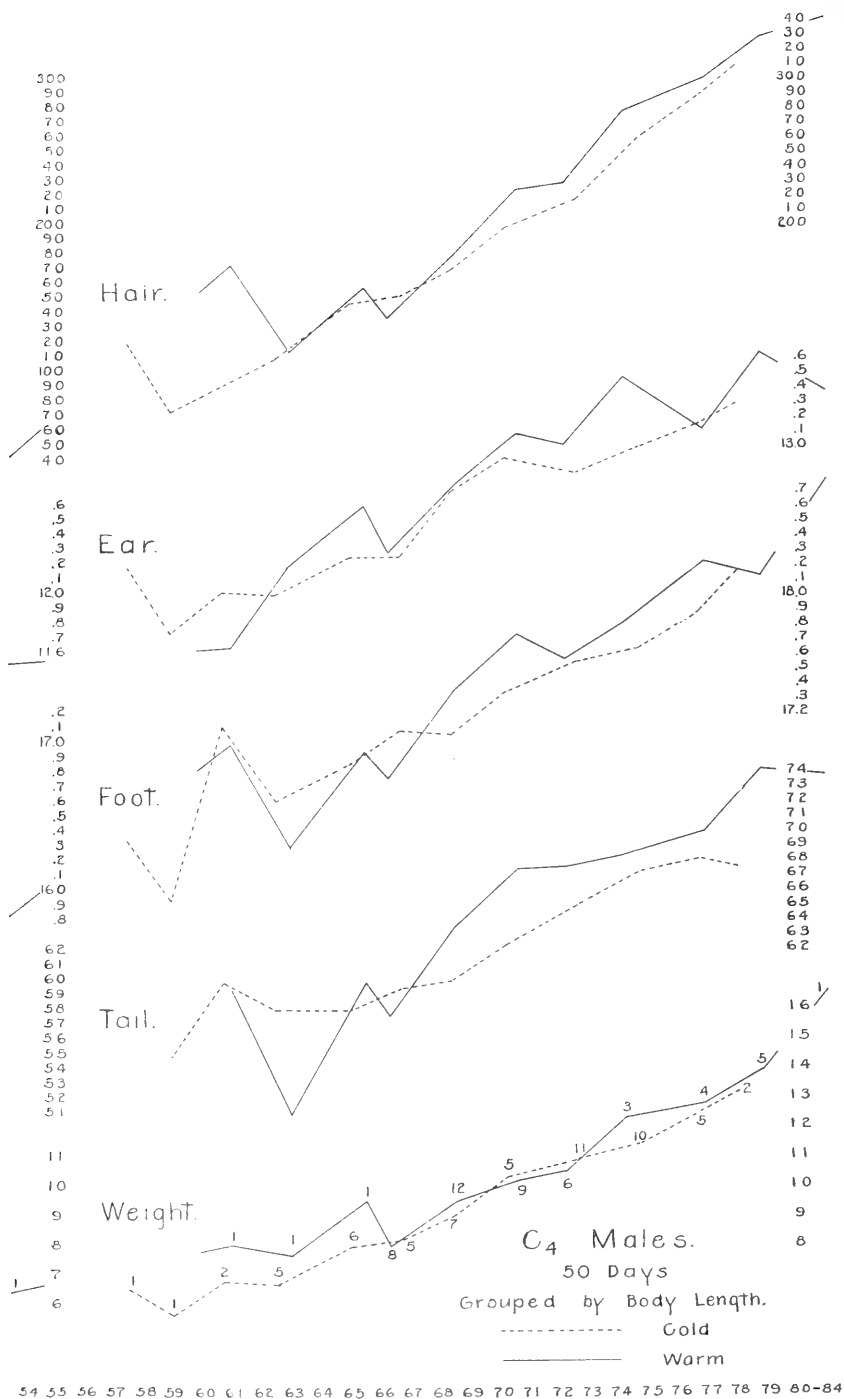


Figure 15

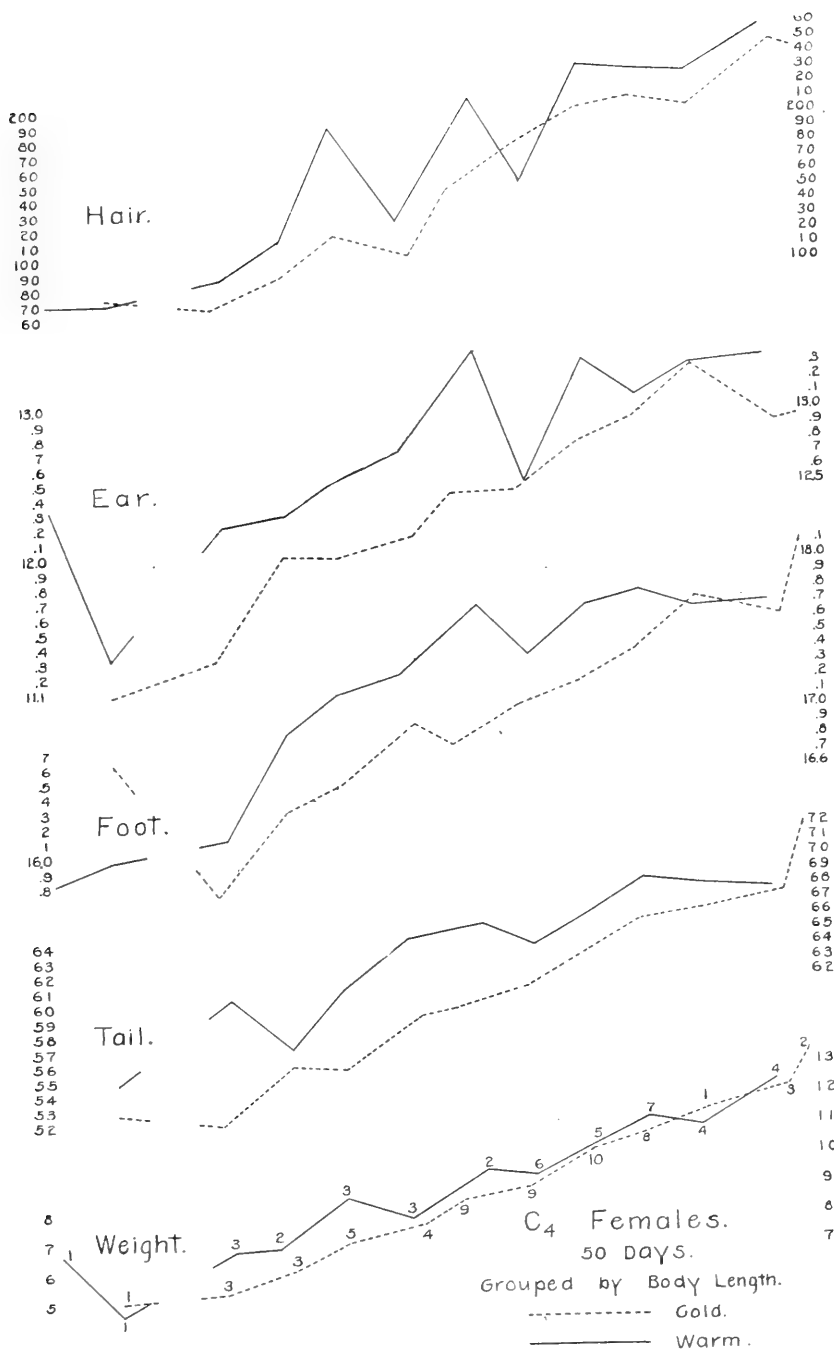


Figure 16
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readily, but he who wishes to examine them for this purpose will find differences between the corresponding sections of two different lots which are in some cases even greater than those between the contrasted sections of the same lot. To take, for example, relative tail length (ratio of tail to body), we have for the males of warm-room parentage, in the C₂, C₃ and C₄ lots, 96.10, 87.59 and 92.41 respectively.³⁰ These differences are in some cases several times (in one instance 14 times) their probable errors, so that, statistically speaking, they are fully as significant as any which we have met with.

Furthermore, in graphs which are not here reproduced, I have compared tail, foot and ear measurements of the corresponding sections of these three lots of mice at the age of 50 days (grouped according to weight). It is immediately obvious that the three curves for the 'cold' or the 'warm' section present, in general, even greater differences among themselves than do the 'cold' and 'warm' curves of the same lot of mice. And the differences between two of these curves are, in many cases, constant throughout their length.

We have, therefore, the same grounds for believing in an actual difference of type between the C₂ and the C₃ or C₄ lot as we have for believing in a difference of type between the 'cold' and 'warm' sections of any one of these lots. And this fact naturally increases the difficulty of interpreting my results. It must be stated, however, that there is no such general agreement in respect to the sign of the differences, for the several characters as was commonly found in comparing the warm-room and cold-room descendants in the same lot. For example, in both sexes, the curve for the C₄ animals of cold-room parentage is the lowermost of the three in the case of tail length, the uppermost of the three for ear length, while for foot length it occupies an intermediate position. And this

³⁰ The figures for the C₃ lot are not quite comparable with the other two, since the measurements on which they are based, were made at the age of 3 months, while the others were made at the age of 50 days. But it is doubtful whether the *relative* tail length differs to any appreciable extent at different ages (pp. 424-425). And furthermore, the C₂ and C₄ figures likewise present considerable differences among themselves.

instance illustrates the generally capricious character of these relations.

It is plain, therefore, that these three lots of mice, born at different times of the same parents³¹ differ from one another unmistakably in their mean characters. But this does not affect the fact that the cold-room and warm-room descendants in all three of these lots differ in the same direction, in respect to weight and ear length, while in two of them the same rule holds for tail and foot length, the third lot showing reverse differences in considerably smaller measure.

The cause of these differences of type between the various C lots is not even suggested in any of my results. An examination of the temperature conditions throughout the entire period of the experiment (figure 1 and table 1) furnishes no clue to the problem.

To express by single averages the relations between the two contrasted sections in these three differing groups of mice is, of course, only partially justifiable. Such averages would be purely fictitious quantities, though this is true, in a sense, of all averages. It may, none the less, serve a useful purpose thus to combine all the animals of cold-room parentage, and likewise those of warm-room parentage, and to find in what measure any general trend emerges, after the cancellation of the differences amongst the various lots.³² Such a general trend is clearly indicated in figure 17 which is based upon the three 'C' broods, combined and divided into size-groups.³³ The relations between the two contrasted series are essentially the same as were previously indicated by the C₂ and C₄ lots, taken separately, and, for one character by the C₃ lot. I do not wish, however, to lay too much stress upon such a composite picture.

³¹ It is, of course, not strictly true that they were born of the *same* parents, since only part of the B₁ lot became parents on each occasion, and the individuals differed in the different cases. This point will be referred to again.

³² This procedure would be unjustifiable unless the proportion of 'warm' and 'cold' individuals were about the same in all three lots.

³³ It has been necessary to use weight groups in the present case, in order to include the C₃ lot for which body length was not determined until later in life.

Averages.
C₂, C₃ and C₄ Lots.
50 Days
Grouped by Weight

----- Cold.
----- Warm.

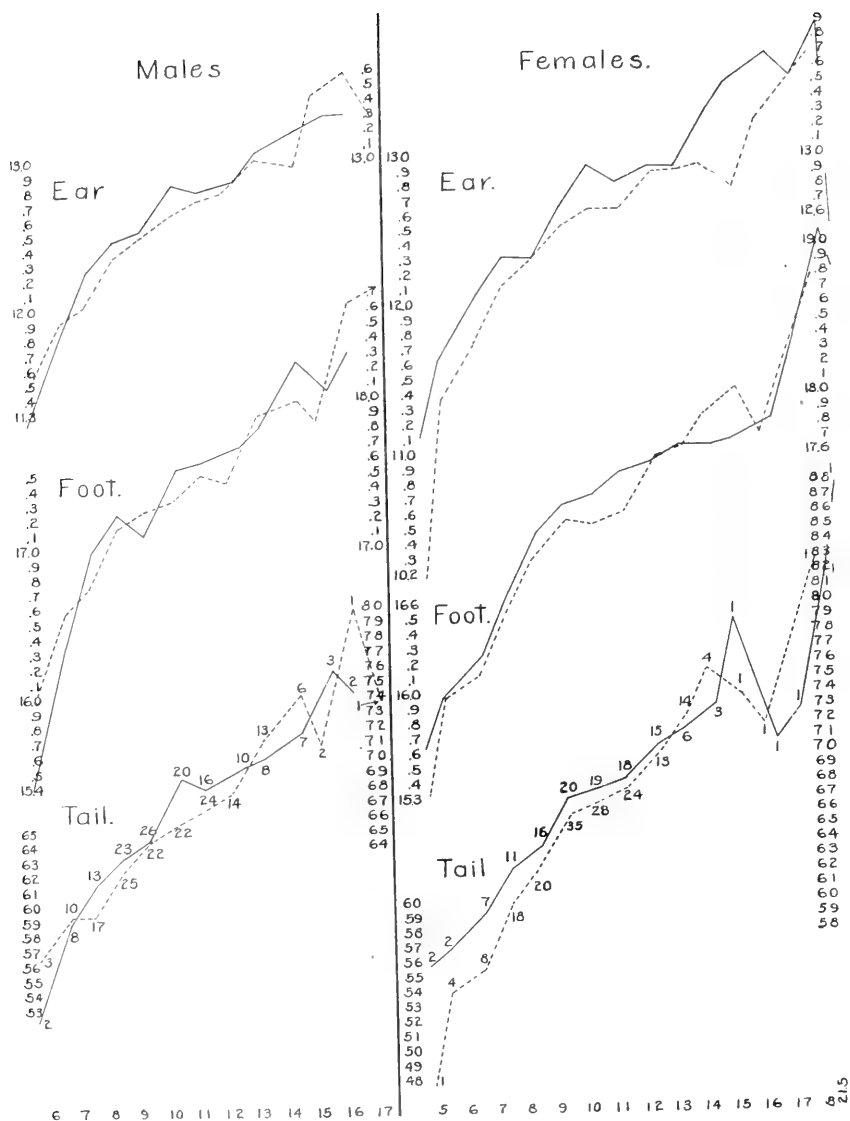


Figure 17

A more reliable expression of the mean tendency of all the F_1 lots, including that of 1909, will be given below.

8. *The 1909 series*

This has already been discussed in three previous papers ('10 a, '10 b, '11), to which I refer the reader for a fuller account of the experiment. In the case of these animals, as already stated, the parents were mated before the discontinuance of the temperature differences, though the entire post-natal life of the offspring, as well as the last five days of their prenatal life, was passed in a common room.

A synopsis of the figures for these mice is given in table 13. The gross averages do not, in some cases, reveal the general tendency of this series, which is shown by the relative tail lengths, and by the mean differences for all the characters, as given in the last section of the table. This general tendency is shown much more clearly by the females than by the males. Some other data were reported in one of my previous papers, where, also, these relations were shown graphically. On the other hand, certain values have here been presented which were not computed previously. The chief point that need concern us at present is the complete agreement in sign between the differences here shown and those discussed above in connection with the C_2 and C_4 lots of the later year.

A CONSIDERATION OF THE F_1 LOTS OF BOTH YEARS' EXPERIMENTS WITH SOME ATTEMPTS AT AN INTERPRETATION OF THE RESULTS

For reasons already stated, I have not thought it worth while to compute gross averages, based upon all the offspring of modified parents which were obtained during my experiments. At least, this has not been done for absolute measurements. It may be of possible interest, however, to state these gross averages for relative tail length. The following figures give the mean values for the ratio of tail to body, based upon the F_1

TABLE 13

1909 mice. 1. Gross averages from measurements made at the age of 6 weeks

		NO.	WEIGHT	TAIL*	FOOT	EAR
			gm.	mm.	mm.	mm.
Of cold-room par- entage.....	Males	71	11.49	72.45	18.033	12.546
	Females	74	10.33	69.61	17.641	12.327
	Both sexes	145	10.90	71.04	17.833	12.434
Of warm-room par- entage.....	Males	75	10.70	70.82	18.011	12.545
	Females	66	10.55	71.61	17.902	12.527
	Both sexes	141	10.63	71.19	17.960	12.536

2. Gross averages from measurements made, after killing, at the age of 3½ months

		NO.	WEIGHT	BODY LENGTH	TAIL ABSO- LUTE	TAIL RELA- TIVE	FOOT	EAR
Of cold-room par- entage.....	Males	60	20.62	89.91	83.45	92.80	18.147	13.483
	Females	51	16.28	83.14	78.62	94.62	17.606	13.178
	Both sexes	114	18.56	86.70	81.16	93.66	17.896	13.339
Of warm-room par- entage.....	Males	46	20.75	89.45	83.06	92.96	18.183	13.550
	Females	38	17.86	85.55	82.33	96.21	18.096	13.471
	Both sexes	84	19.45	87.68	82.73	94.43	18.143	13.514

3. Mean differences (warm—cold) from measurements at the age of 3½ months;
(grouped according to body length)

	WEIGHT	TAIL ABSOLUTE	FOOT	EAR
Males.....	+0.47	+0.73	+0.068	+0.086
Females.....	+0.23	+1.52	+0.282	+0.096

* These tail measurements, being made upon living mice, are not quite comparable with those given in most of the other tables; (see p. 340).

TABLE 14

Mean differences (warm—cold) for all the offspring of modified parents; (grouped according to body length)

	LOT	$N_c \times N_w$	WEIGHT	TAIL	FOOT	EAR
			gm.	mm.	mm.	mm.
Males.....	C ₂	1364	+0.14	+2.48	+0.390	+0.039
	C ₃	1932	+0.57	-0.38	-0.022	+0.200
	C ₄	3120	+0.25	+2.46	+0.137	+0.142
	1909 lot	2760	+0.47	+0.73	+0.068	+0.086
	Grand averages		+0.367	+1.345	+0.1204	+0.1221
Females....	C ₂	1700	+0.21	+2.39	+0.261	+0.061
	C ₃	2268	+0.30	-1.77	-0.075	+0.227
	C ₄	2378	+0.51	+3.15	+0.429	+0.359
	1909 lot	2052	+0.23	+1.52	+0.282	+0.096
	Grand averages		+0.324	+1.269	+0.2230	+0.1988

lot of 1909, and the three F₁ lots (C₂, C₃ and C₄) of 1911, a total of 752 mice:³⁴

	Males	Females
Cold-room descendants.....	90.87	92.07
Warm-room descendants.....	91.94	93.79

The 'warm' figures are seen to be from 1 to 1 $\frac{3}{4}$ per cent greater than the 'cold.'

Another more legitimate way of comparing the aggregate cold-room and warm-room descendants, in respect to a given character, is to obtain not the mean value of that character itself for all the animals in each of the two contrasted series ('cold' and 'warm'), but the mean of the various differences between these series which were obtained for the separate lots of animals by the method of size-groups. In table 14 are given first the mean differences for the C₂, C₃, C₄ and 1909 lots, derived from tables 8, 11, 12 and 13, and then the weighted means of these averages (pp. 345-346). It will be seen that the differences are all of the same sign, with the exception of those for tail and foot in the C₃ lot (both male and female).

³⁴ Since the later measurements of the C₃ and the 1909 lots are here considered, this number is considerably less than the total number measured earlier in life. In all, 879 F₁ mice were actually measured. Of course the B₃ lot (p. 371) is not included in these computations.

In the latter cases, the sign is reversed, though the differences are much smaller in amount than the positive ones shown by the other three lots. As corroborative evidence, of possible relevance, we have the differences between the C₂ and B₃ lots, which were shown in table 9.

These figures, in the aggregate, seem to me to render it highly probable that the two contrasted series of mice differed from one another because they were the offspring of cold-room and warm-room parents, and not because of chance assortment or of any irrelevant influences acting upon the animals during the experiments. This may be said without committing myself to any particular interpretation of the facts. Whatever differences in type the four different lots display among themselves, they all agree with one partial exception, in showing a greater body weight, and greater length of tail, foot and ear for the offspring of warm-room parents. I freely grant that the single partial exception, among the four cases, considerably weakens the statistical significance of the figures. Likewise the fact that greater differences are sometimes shown between corresponding sections of different lots than between the two contrasted sections of the same lot point to the operation of wholly unknown causes which affect to a considerable degree the growth of the body parts under consideration. For the C₂, C₃ and C₄ lots were the offspring of approximately the same groups of parent animals, and the conditions to which they were exposed during growth, so far as temperature, at least, is concerned, do not satisfactorily account for any of the differences between them.

I say *approximately* the same groups of parent animals, since precisely the same individuals among the B₁ mice do not figure as parents of the various C lots. It might therefore be suggested that these accidental differences in the parentage of the different 'C' (i.e., F₁) lots might account for the differences which they display among themselves. And, as I have already pointed out, the possibility of original germinal differences between the cold-room and warm-room sections of the parent generation might be suggested as an explanation of the differences shown by the two contrasted lots of offspring.

In table 15 those members of the A generation have been grouped by themselves which became the parents of the B₁ and B₃ groups respectively, as well as the grandparents of each of the three C lots. Only the figures for weight and tail length are here available. In table 16 the B₁ parents have been similarly grouped in relation to the several C lots.³⁵ While such somatic differences as are here indicated bear no necessary relation to differences in germinal constitution, they should, in the aggregate, reveal the presence of such differences if they occurred.

TABLE 15

'A generation' mice; parents and grandparents of various lots; based upon measurements of living mice, October, 1910

				NO.	WEIGHT	TAIL
					gm.	mm.
Parents of B ₁ lot.....	Males	cold		22	23.97	82.98
		warm		22	24.46	83.26
	Females	cold		39	23.81	89.63
		warm		51	23.31	89.66
Parents of B ₃ lot.....	Males	cold		18	24.38	85.27
		warm		10	22.98	82.48
	Females	cold		31	24.28	89.93
		warm		16	23.86	89.59
Grandparents of C ₂ lot....	Males	cold		16	23.87	81.51
		warm		14	23.70	83.11
	Females	cold		23	23.83	90.60
		warm		21	24.17	90.80
Grandparents of C ₃ lot.....	Males	cold		17	23.76	83.64
		warm		19	24.78	83.80
	Females	cold		27	23.95	89.50
		warm		29	23.01	89.84
Grandparents of C ₄ lot.....	Males	cold		18	23.50	82.80
		warm		18	24.10	82.40
	Females	cold		26	24.29	90.19
		warm		24	23.67	90.50

³⁵ These various means are not merely the simple averages of the characters for the respective groups of parents. In computing them the measurements for each parents were weighted in accordance with its number of offspring.

TABLE 16

B₁ mice; parents of the several C lots, arranged for comparison; based upon the final measurements of the mice, August and September, 1911

			NO.	WEIGHT	BODY	TAIL	FOOT	EAR
				gm.	mm.	mm.	mm.	mm.
Cold...	Males	parents of C ₂ lot	18	22.30	93.73	78.86	17.368	14.110
		parents of C ₃ lot	19	23.13	95.70	81.42	17.370	14.347
		parents of C ₄ lot	18	23.44	96.12	81.19	17.453	14.177
	Females	parents of C ₂ lot	25	21.25	94.79	78.39	17.217	14.341
		parents of C ₃ lot	30	21.82	95.19	79.87	17.129	14.169
		parents of C ₄ lot	28	20.35	94.02	79.55	17.214	14.308
Warm .	Males	parents of C ₂ lot	16	24.42	95.22	84.74	17.759	14.433
		parents of C ₃ lot	18	24.06	94.42	84.61	17.616	14.156
		parents of C ₄ lot	15	24.83	95.95	85.70	17.699	14.317
	Females	parents of C ₂ lot	21	23.35	97.11	86.58	17.637	14.216
		parents of C ₃ lot	28	23.55	96.14	85.05	17.883	14.369
		parents of C ₄ lot	24	21.77	95.26	86.41	17.607	14.298

Of course no original germinal differences between the cold-room and warm-room sections of the B₁ lot could here be recognized, since they would have been overlaid by the effects of differing environment. But here we may perhaps learn something from a consideration of the grandparental (A) generation. On the other hand, this grouping of the B₁ animals might be instructive in relation to the differences shown by the C lots among themselves.

I can hardly expect the reader to make any careful study of these two tables (15 and 16). I have myself analyzed them with some care and can find in these chance differences of parentage no satisfactory explanation of any of the conditions found in the 'C' generation. For, although the differences are in some cases of the sort required by such an explanation,³⁶ (1) they are in some cases of an exactly reverse nature, and (2) they are in no cases of sufficient magnitude to have any bearing on the situation. They are commonly even smaller than the differences found in the C generation, whereas a consideration

³⁶ Of course the differences between the warm-room and cold-room animals of the B₁ generation are not here under consideration.

of the coefficient of heredity (pp. 420-421) shows us that even in the parent generation, they should be several times as large as these, in order to have any explanatory value in this connection.

Another objection may be offered to the significance of my results, namely that my statistical procedure has led me to underestimate the operation of chance in determining these differences between the two contrasted lots in the F_1 generation. I have, it may be pointed out, used individuals as units. In order to determine whether the relations which I had found when individuals were used as units would be altered if fraternities were taken instead, the figures shown in table 17 were computed. It will be seen that, for relative tail length, the sign of the difference between the 'warm' and 'cold' figures has in no case been changed, and that, in general, the averages computed by the two methods are closely similar. I have taken the trouble to obtain the probable errors for the C_2 males only. These are considerably greater (24 to 35 per cent) when fraternities are used as units, and this difference would doubtless hold throughout the table. But such changes would not seriously weaken the validity of my results.

TABLE 17

Comparison of averages based upon brood means with averages based upon individuals taken separately; ratio of tail to body

				NUMBER OF INDIVIDUALS	NUMBER OF FRATERNITIES	AVERAGES BASED UPON INDIVIDUALS	AVERAGES BASED UPON FRATERNITIES
C_2 lot...	Males	cold		41	22	91.86 \pm 0.58	92.59 \pm 0.72
		warm		31	12	96.10 \pm 0.48	95.82 \pm 0.65
	Females	cold		50	23	93.10	93.51
		warm		34	19	96.39	96.40
C_3 lot...	Males	cold		42	24	88.81	88.50
		warm		46	21	87.59	87.97
	Females	cold		54	24	90.42	90.72
		warm		42	20	88.57	88.54
C_4 lot...	Males	cold		60	25	89.66	89.64
		warm		52	22	92.41	92.62
	Females	cold		58	27	90.34	90.46
		warm		41	20	94.74	95.38

Another fact which more seriously affects the interpretation of my results must now be considered. Mention was earlier made of the fact that about a third of the warm-room section of the parent (B_1) mice were kept for two weeks in the cold-room, before being transferred to the warm-room. It is well known that, whereas adult mammals maintain a nearly or quite constant temperature under the most diverse atmospheric conditions, the young of many species pass through an undeveloped stage during which their body temperature may fluctuate widely in accordance with external conditions. It might therefore be argued that even if the germ-cells could not have been directly influenced during the adult life of the parents by the temperature conditions imposed upon the latter, they may have been so influenced during those early days before homothermy had been established.

Unfortunately, the number of individuals available for this highly important test was very limited. Out of the 262 mice of warm-room parentage in the C_2 , C_3 and C_4 lots, only 72 were the offspring of parents which had been kept in the cold-room during the earlier weeks of their lives. There are thus, on the average, only 12 individuals in each of the six groups comprised in table 18. But it is of possible significance that in each of these six cases, the figure indicating relative tail length is lower for animals of this parentage than for the warm-room descendants at large.³⁷ The small number of individuals available renders a comparison by size-groups impracticable, and we have seen that absolute measurements can be compared only with great caution. We find, however, that in respect to tail, foot and ear, the offspring of the 'b' section show a lower absolute figure in 13 out of 18 cases than those of the warm-room parents at large. Since, however, the mean body length likewise differs, this comparison is not very instructive. It must be stated, finally, that these figures for the offspring of

³⁷ A fairer comparison would have been that between the offspring of the 'b' section of the warm-room parents, and those of the 'a' section alone (table 2). The contrasts thus shown would, of course, have been even greater.

the warm-room 'b' series have, in some cases, actually fallen below those for the animals of cold-room parentage.³⁸

I have computed the mean relative tail length for such of the 'b' section of the B₁ warm-room mice as became parents. In the case of the males, this figure agrees very well with that for the warm-room parents at large, while in the female lot it is actually greater. The relations shown by the young do not seem, therefore, to be explained by any measurable parental differences.

These meager data, based upon such small numbers of animals, are, of course, inconclusive. But in so far as they have any significance at all, they point to the possibility that the greater part of the modification of the germ-cells, from which resulted the differences between my 'warm' and 'cold' series in the F₁ generation, was effected during the first two weeks of parental life, at a time when the body was partially permeable to temperature influences. This fact, if true, would be in conformity with the current explanation, offered by opponents of the Lamarckian principle, for the various known cases in which parent and offspring have been modified in a parallel manner, namely that the given environmental agencies have affected soma and germ-cells simultaneously. And I must concede that the very inadequate data of my own, just cited, lend more probability to this alternative than I was previously disposed to grant it. As I have more than once declared, however, this hypothesis of "simultaneous modification of the germ-cells" is by no means as simple as it looks, and, in respect to the 'conceivability' of the processes involved, it has little advantage over theories of a more strictly Lamarckian type. This is particularly true if the chief factor in bringing about the parental modifications was humidity, rather than temperature, for at no time can the body be said to be permeable to atmospheric humidity.

It must now be considered in how far the modifications of parent and offspring in my experiments were actually *parallel*.

³⁸ That is, in addition to those cases (in the C₃ lot) where the 'warm' figures were already lower.

For tail and foot length, this was plainly true, though the degree of modification was greatly reduced in the second generation. As regards weight and ear length, however, the case is much less evident, and some attention may therefore be given to them at this point. By weight I here refer to relative weight, as seen in comparing animals of the same body length. As regards absolute weight, there would seem to be no general law, either in parents or offspring. It was found that in the parent (B_1) lot of 1910–1911, both in the males and females, the relative weight was greater for the warm-room animals (pp. 365–368). This condition agrees, therefore, with that found in all of the lots of offspring (C_2 , C_3 and C_4). The condition, in this respect, of the parents of the 1909 lot, cannot be stated, since their body length was not determined. In the results of an earlier year (1907–1908), the figures for the two sexes contradict one another, and it has also been seen that in the case of those males of 1911 which were killed at the age of 4 months, the cold-room individuals were the heavier.

While, therefore, we can find no evidence of any such general agreement in the effects of temperature upon body weight as was found in the case of tail and foot length, it is of possible significance that a lot which comprised the parents of three-fourths of the F_1 offspring here discussed showed, in both sexes, the same differences as their young.

The existence of any constant effect of temperature upon the ear length of the parent generation is even less certain than that upon body weight. In the experiments of 1910–1911, we certainly have no good evidence for such an effect. The figures in tables 4, 5 and 6 contradict one another, and in no case are the differences large enough to be significant. Slight indications of a greater ear length for animals reared in the warm-room were noted during the experiments of an earlier year (1907–1908), and a seemingly convincing case was observed in the spring of 1909. Graphs for this last are shown in one of my previous papers ('10 b, fig. 4). This last is the only case having any considerable statistical certainty, but it is to be noted that we are here dealing with *animals whose parents had*

likewise been subjected to temperature differences. It is not altogether certain, therefore, that even here these differences in ear length resulted from the direct effect of temperature conditions upon the animals after birth.

It is true that some writers have pointed out a difference in ear length between northern and southern races of certain mammals, or between desert forms and those inhabiting a more humid climate (see my paper of 1909). While this fact is highly suggestive, it has never been shown that these differences resulted from the direct effect of environmental conditions acting in a single lifetime.³⁹

Despite the lack of any certain modifications of ear length in the parent generation, differences in the mean length of this organ were shown by the offspring of warm-room and cold-room parents, which were of about the same magnitude as those shown for foot length. Indeed, it is seen in table 14 that these differences in ear length are of the same sign, without a single exception, for both sexes and for all four of the F_1 lots of mice. This degree of unanimity is not found in the case of either tail or foot.

It may with justice be urged that we can have no inheritance of an "acquired character," when, the character in question is not actually acquired, or even evinced, by the parents themselves. And, indeed, this case of the ear length of my F_1 mice tells rather heavily against the view that the peculiarities of this generation resulted from any specific influence exerted by the modified parts of the parent body upon the corresponding parts of the offspring.

The subject of correlation, to be discussed more fully later, still further complicates the present problem. For, as will be shown below, all four of the characters in respect to which the cold-room and warm-room descendants differ from one another are positively correlated, at least in young mice. This fact gives some support to the view that these various differences in structure between the two contrasted lots of animals

³⁹ I have in progress some experiments with wild mice of the genus *Peromyscus* which, I hope, will throw some light upon this and related subjects.

are all merely expressions of some general constitutional difference. And the transmission of such general constitutional differences, even when individually acquired, is hardly in doubt.

But whatever interpretation be given to these results, the central fact must not be overlooked that the offspring of two groups of parents which had been modified in divergent directions by differences of atmospheric temperature and humidity, presented certain characteristic differences, and that some, at least, of these differences agreed with those shown by the parents, in sign, if not in amount. Little light has been thrown upon the mechanism by which this repetition of parental differences has been brought about, but there are circumstances which do not favor the view that we have to do with a specific (part-for-part) influence of soma upon germ. There may, none the less, have been an influence of a sort capable of acting in a cumulative manner from generation to generation, and of producing extensive racial modifications.

As often happens in a research of this kind, various incidental results or by-products have been obtained, which have no bearing on the main issues under consideration, but which are none the less of interest. Some of these will be discussed in the following sections.

VARIABILITY

I have given very little attention, in these studies, to the subject of variation, as such. Standard deviations have been computed, to be sure, but this has been done chiefly as a step to the computation of probable errors and of correlation coefficients. These standard deviations have been included in many of the preceding tables. In table 19, I have given the averages of the standard deviations obtained for the same characters in the various series of animals. Since it was thought that the variability of the younger animals measured might differ from that of the older ones, a separation into older and younger lots has been made. Under each of these heads two sets of figures have been given. One of these represents the average variability of the various characters in populations heterogeneous

TABLE 19

Standard deviations of measurements, representing variability, both in general population, and within groups homogeneous in respect to size

		WEIGHT	BODY LENGTH	TAIL ABSO- LUTE	FOOT	EAR
		gm.	mm.	mm.	mm.	mm.
Younger animals (50 days).	Means of standard deviations, based upon entire lots*	2.385	5.522	5.336	0.6386	0.5375
	Means of standard deviations, based upon size-groups†	0.872	0.518	3.421	0.4811	0.3474
Older ani- mals (3 months to fully ma- ture).....	Means of standard deviations, based upon entire lots‡	2.893	3.872	4.870	0.5915	0.4956
	Means of standard deviations, based upon size-groups§	1.566	0.514	3.416	0.4729	0.4418

* B₃, C₂, and C₄ lots, comprising 527 individuals, here included, the warm and cold-room sections, and the males and females, being taken separately, as in the foregoing tables.

† Seven groups from the lots last named, comprising 76 individuals, here included.

‡ A, B₁ (superfluous males and adult females) and C₃ (at 3 months) lots, comprising 540 individuals, here included.

§ Fifteen groups from the lots last named, comprising 229 individuals, here included.

in respect to size. These figures are merely the (weighted) means of the standard deviations given in the previous tables. The other set of figures consists of the averages of the standard deviations for size-groups, i.e., they represent variabilities within groups nearly homogeneous in respect to size.⁴⁰ The latter figures are, as might have been expected, in all cases smaller than the former. But the differences (save for body length, on which the size-groups are based) are much less than one might perhaps have predicted. The greater general variability of the younger animals, as compared with the older, is seen to result mainly from the greater variability in body length.

⁴⁰ Two-millimeter intervals were used in grouping the animals. Only those size-groups were included in the present computations which contained ten or more individuals.

CORRELATION

Reference has already been made (pp. 348-350) to the two types of correlation which may be distinguished. In the one case, we may obtain correlation coefficients for general populations, i.e., for masses which are heterogeneous in size. Correlations between two characters, as thus determined, are partly, or even wholly, due to the fact that both characters are correlated with general size of body. In the second, we may obtain correlation coefficients for groups which are homogeneous in respect to body length. Correlations thus determined obviously have a much greater biological interest than those of the first class named.

In table 20 are given coefficients between every two characters which were measured, obtained in the first way stated. Relatively few of the animals were included in these computations, since the task is a very laborious one and the results, I believe, are of secondary biological interest. It will be seen that, with a single exception, all of the separate coefficients obtained are positive, while in the one exceptional case the coefficient is practically zero. The means of the columns are in all cases positive, ranging from 0.133 to 0.800. The grand average is 0.429, a figure which is lower than most of those given by Pearson ('00, p. 402) for man.

Despite the small numbers of individuals concerned, the relative magnitudes of some of my figures are doubtless significant. For example, the greatest degree of correlation found was that between weight and body length.

The correlations which were found to obtain within groups of individuals of approximately the same size are presented in tables 21 and 22.⁴¹ Animals of nearly all of my experimental lots were included in these computations. It was soon noted that, in respect to certain of the cases of correlation, the younger animals showed on the whole marked differences from the

⁴¹ This grouping is a little arbitrary in certain cases. The C₃ and the 1909 mice (3 to 3½ months old) have been included in the 'immature' group, while the small number of 'superfluous males' of the B₁ lot (4 months old) were included in the older group.

TABLE 21

Correlations between characters in groups of individuals of approximately the same size (2-mm. intervals). A and B₁ mice

LOT	SIZE-GROUP	NO.	TAIL AND WEIGHT	FOOT AND WEIGHT	EAR AND WEIGHT	HAIR AND WEIGHT	FOOT AND TAIL	EAR AND TAIL	HAIR AND TAIL	EAR AND FOOT	HAIR AND FOOT	EAR AND HAIR
A males....	94-95	13	+0.08	+0.89	+0.47		+0.18	-0.07		+0.52		
A females...	92-93	12	-0.53	-0.08	-0.04		+0.24	-0.42		-0.01(-)		
A females...	94-95	22	-0.59	+0.08	+0.14		+0.02	-0.11		+0.17		
A females...	96-97	22	-0.37	+0.26	+0.36		+0.02	-0.35		+0.68		
A females...	98-99	29	-0.04	+0.38	-0.16		-0.16	-0.18		+0.64		
A females...	100-101	19	-0.11	+0.25	+0.07		-0.36	-0.45		+0.43		
B ₁ superflu- ous males (warm)...	86-87	12	-0.16	+0.24	+0.45	+0.62	+0.42	-0.21	+0.06	+0.50	+0.27	+0.53
B ₁ females (cold)...	94-95	14	-0.48	-0.28	-0.02	+0.07	+0.59	+0.09	+0.04	+0.70	+0.01	+0.12
B ₁ females (warm)...	94-95	12	-0.25	+0.47	+0.51	+0.18	+0.45	+0.12	+0.05	+0.65	-0.33	-0.02
B ₁ females (warm)...	96-97	15	-0.22	+0.03	-0.24	-0.15	+0.71	+0.22	+0.20	+0.35	+0.32	+0.14
B ₁ females (warm)...	98-99	12	-0.38	-0.25	-0.42	+0.04	+0.42	-0.12	-0.07	+0.46	-0.03	+0.20
Mean....		182*	-0.268	+0.199	+0.090	+0.132	+0.166	-0.151	+0.060	+0.481	+0.052	+0.185

* Figures relating to hair are based on only 63 individuals.

TABLE 22
Correlations between characters in groups of individuals of approximately the same size (2-mm. intervals). Immature mice

LOT	SIZE-GROUP	NO.	TAIL AND WEIGHT	FOOT AND WEIGHT	EAR AND WEIGHT	HAIR AND WEIGHT	FOOT AND TAIL	EAR AND TAIL	HAIR AND TAIL	EAR AND FOOT	HAIR AND FOOT	EAR AND HAIR
B ₃ males (cold).....	70-71	12	+0.16	+0.30	+0.69		+0.42	+0.10		+0.54		
C ₂ males (cold).....	72-73	11	-0.21	-0.55	+0.29		+0.82	+0.18		+0.08		
C ₂ females (cold).....	70-71	10	+0.05	+0.07	+0.35		+0.91	+0.66		+0.82		
C ₃ males (cold).....	84-85	12	-0.46	+0.32	+0.16		+0.19	-0.14		+0.43		
C ₃ females (cold).....	82-83	12	+0.42	+0.59	+0.36		+0.24	+0.30		+0.83		
C ₃ females (cold).....	84-85	13	+0.21	+0.05	+0.15		+0.44	+0.51		+0.75		
C ₃ females (warm)....	80-81	10	+0.34	+0.66	+0.75		+0.72	+0.58		+0.87		
C ₄ males (cold).....	72-73	11	-0.16	-0.20	+0.32	+0.16	+0.84	+0.29	+0.44	+0.35	+0.67	+0.65
C ₄ males (cold).....	74-75	10	+0.40	+0.29	+0.47	+0.48	+0.66	+0.64	+0.58	+0.57	+0.79	+0.52
C ₄ females (cold).....	70-71	10	-0.04	-0.18	-0.17	-0.28	+0.80	+0.76	-0.66	+0.75	-0.50	-0.69
C ₄ males (warm)....	68-69	12	+0.51	+0.16	+0.75	+0.72	+0.66	+0.52	+0.21	+0.42	+0.28	+0.71
1909 males (cold).....	88-89	11	-0.43	+0.07	+0.42		+0.54	+0.27		+0.78		

1909 males (cold).....	90-91	13	-0.16	-0.20	+0.09	+0.42	+0.21	+0.11
1909 males (cold).....	92-93	12	+0.24	+0.33	+0.53	+0.40	+0.00	+0.55
1909 females (cold).....	80-81	13	+0.59	+0.46	+0.44	+0.70	+0.80	+0.88
1909 females (cold).....	82-83	16	+0.21	+0.10	+0.19	+0.79	+0.45	+0.71
1909 females (cold).....	84-85	14	+0.46	+0.60	+0.23	+0.74	+0.36	+0.84
1909 males (warm)...	88-89	15	+0.64	+0.12	+0.16	+0.51	+0.37	+0.35
1909 females (warm)...	84-85	13	-0.02	+0.13	+0.35	+0.42	+0.44	+0.49
Mean...		230* (Total number)	+0.159	+0.164	+0.336	+0.302	+0.380	+0.165
							+0.336	+0.349

* Figures relating to hair are based on only 42 individuals.

older ones. The figures for the two groups have therefore been separated into two tables.

A comparison between tables 20 and 21 is highly instructive, though we must make this comparison with some caution. For the series of animals comprised in the two tables are not identical. As already stated, only those size-groups have been considered in my computations which contained ten or more individuals. Thus, about 100 of the animals included in the first table have been excluded in the second.⁴²

It will be noted at once that in every single case, in which comparison is possible, these values in the second table are lower than those of the first table. Indeed in two cases (those of tail and weight and of tail and ear) the sign has been reversed. In these two cases, particularly the first, the high proportion of negative figures in the respective columns indicates a probable significance for the sign of these averages. If we compute the mean value of these correlation coefficients in the second table and that for the same ten coefficients in the first table, we have $+0.095$ and $+0.371$, respectively. In other words the correlations which were found among animals of approximately the same size were scarcely more than a fourth of those obtained when general populations were considered irrespective of size. Of course the numbers of individuals which I have used are so small that we cannot rely upon any such exact ratios, but the overwhelming preponderance of one figure over the other cannot be accidental. Indeed, a considerable difference between these two figures is to be regarded as a mathematical necessity.

Passing to table 22, giving correlation coefficients for the younger animals after division into size-groups, we meet with the rather surprising fact that the correlations shown by these younger mice are very much higher than those shown by the older ones. Likewise, the mean values are now in all cases posi-

⁴² We must also mention the (perhaps unfortunate) inclusion of the 'superfluous males' of the B_1 lot, which were not previously taken into account, but which are included in table 21. There are, however, only 12 of these animals, so that they could have little effect in determining the averages.

tive. The grand average of the ten coefficients is here 0.336, a figure between 3 and 4 times as great as that for the older animals, when the latter were similarly divided into size-groups, and indeed not far below that for the older animals when treated as general populations, irrespective of size. I have shirked the immense task of obtaining the correlation coefficients for all these series of younger animals, according to the other method of computation. But this has been done for one lot, the C₄ lot, which affords a good basis of comparison between the correlation coefficients obtained for the younger animals, according to the two methods. In table 23, the coefficients are given separately for the 'warm' and 'cold' sections and for the two sexes of the C₄ lots; likewise the averages for these four groups. It will be readily seen that the coefficients are very much higher than in table 22. The mean of all the figures in table 23 is 0.737, as compared with 0.336, when the animals were divided into size-groups. The latter figure, it is true, is the mean for all the series of younger mice. The C₄ lot is represented in table 22 by only four small size-groups, comprising 43 individuals. The mean of the coefficients for these alone is 0.344, a figure which agrees pretty closely with that for table 22 as a whole, and is less than half the mean for table 23.

Summarizing our data for intra-individual correlation, we may say (1) that the coefficients are from two to four times as great when they are based upon general populations as when they are based upon groups of approximately equal size; and (2) the younger animals show a much higher correlation (computed by either method) than do the older ones. Indeed, certain coefficients which are positive for the former are negative for the latter. The meaning of the second fact is quite unintelligible to me at present.

Another type of correlation, which I have treated in a very inadequate way, is that between parents and offspring. The measure of this has been termed by Pearson the "coefficient of heredity." For reasons already stated (pp. 350-351) the only character for which I have computed "coefficients of heredity" is relative tail length (ratio of tail length to body length).

TABLE 23
Correlations among young mice (C₄), based upon undivided series, containing individuals of diverse size

	NO.	TAIL AND WEIGHT	FOOT AND WEIGHT	EAR AND WEIGHT	HAIR AND WEIGHT	FOOT AND TAIL	EAR AND TAIL	HAIR AND TAIL	EAR AND FOOT	HAIR AND FOOT	EAR AND HAIR
Cold males.....	60	+0.67	+0.68	+0.82	+0.83	+0.79	+0.65	+0.71	+0.70	+0.77	+0.76
Cold females.....	58	+0.78	+0.74	+0.70	+0.78	+0.86	+0.83	+0.62	+0.79	+0.66	+0.67
Warm males.....	52	+0.74	+0.71	+0.78	+0.86	+0.79	+0.76	+0.79	+0.77	+0.73	+0.79
Warm females.....	41	+0.66	+0.70	+0.73	+0.83	+0.51	+0.59	+0.53	+0.73	+0.80	+0.74
Mean.....	211	+0.716	+0.708	+0.760	+0.824	+0.755	+0.715	+0.670	+0.748	+0.736	+0.739

Here again, I have employed two different methods in computing the coefficients, and with unlike results. In the first place (table 24), the parents of each sex have been thrown together to form single populations. That is, the cold-room and warm-room lots have been combined, and the averages and deviations obtained for these aggregations. The same has been done for the offspring. Not only have those of cold-room and warm-room parentage been averaged together, but those belonging to the C_2 , C_3 and C_4 lots. The sexes, however, both among parents and offspring, have been treated separately.

Objection may be made to this method of treatment, on the ground that it is not legitimate to combine as single populations animals which differ as widely from one another as the cold-room and warm-room sections of the parent lot. In fact, the occurrence of an apparent "correlation due to heterogeneity of material" can be mathematically demonstrated (see Yule '11, pp. 214-215). The validity of this objection is in large

TABLE 24

*"Coefficients of heredity" (filial-parental correlation) based upon relative tail length. (In the computation of these coefficients, the entire B_1 lot, 'cold' and 'warm,' has been treated as a single population. Likewise the three C lots have been averaged together, without distinction as to 'cold' or 'warm' parentage. The sexes have, however, been treated separately)**

	NUMBER OF OFFSPRING	COEFFICIENT
Male parents and male offspring.....	261	+0.105±0.041
Male parents and female offspring.....	266	+0.200±0.040
Female parents and male offspring.....	258	+0.229±0.040
Female parents and female offspring.....	250	+0.268±0.040
Mean.....		+0.200

* As already stated, I have, for reasons of expediency, used all the offspring of a brood, *taken individually*, as units in computing these coefficients. The more usual and justifiable procedure is to use either a single member of each brood, chosen arbitrarily, or the mean of each brood. Thus the figures here given are not quite comparable with similar coefficients which have been obtained by others. But they are strictly comparable with those given in the next table, which is the essential thing for our present purposes. For the reasons just mentioned the probable errors of this table have not the same significance as those ordinarily obtained, being doubtless too low.

degree conceded, and I hasten to explain that this first method of computing the correlation between parents and offspring has been employed only for the purpose of comparison with a second, more justifiable method. We may ask, however, whether the procedure which I have adopted in the first case is not that which is ordinarily employed in computing "coefficients of heredity."⁴³ Individuals of very diverse environmental history (which might readily be divided into two or more groups with varying means) are lumped together as a single 'population,' and correlations are determined between these and their offspring. Now these parents display a variability of two-fold origin. An important part of it is, of course, of germinal origin. But another part has none the less certainly been due to differences of environment, acting during growth. In cases where there is a tendency for the offspring to be subjected to the same environmental influences as the parents, it can hardly be doubted that a part of the correlation found to obtain between them is due to parallel somatic modifications. In my experiments, however, parents of two groups were exposed to diverse environmental influences, while the offspring, without regard to parentage, were exposed to identical influences, so far, at least, as these specific factors were concerned. Accordingly, if the offspring of the two contrasted lots tend to diverge in the same direction as their parents, the correlation coefficients, computed as above indicated, will be larger than they will be when the cold-room and warm-room sections are treated as separate populations. For, by the latter method of treatment, the coefficients will express, much more nearly, the correlation with respect to characters which were of germinal original in the parents, while by the former method, correlation in respect to the induced differences will likewise figure in the results.

A comparison of tables 24 and 25, based upon the two methods of computing these coefficients, shows at once that this expectation is realized. Not only is the grand average greater in the former case than the latter (0.200 and 0.142 respectively)

⁴³ Save for the fact indicated in the foot-note to table 24.

TABLE 25

"Coefficients of heredity" (filial-parental correlation); based upon relative tail length. (Here the 'cold' and 'warm' sections, both of parents and offspring, have been treated separately, and the three C lots have not been combined).

		MALE PARENTS AND MALE OFFSPRING		MALE PARENTS AND FEMALE OFFSPRING		FEMALE PARENTS AND MALE OFFSPRING		FEMALE PARENTS AND FEMALE OFFSPRING	
		Num- ber of cases	Coefficients	Num- ber of cases	Coefficients	Num- ber of cases	Coefficients	Num- ber of cases	Coefficients
C ₂ ..	cold	42	+0.34	48	+0.29	41	+0.30	40	+0.31
	warm	30	-0.23	33	+0.06	29	+0.01	32	-0.13
C ₃ ..	cold	40	+0.22	52	+0.29	40	+0.17	46	+0.15
	warm	46	+0.24	40	+0.27	41	+0.01	35	+0.30
C ₄ ..	cold	52	-0.12	54	+0.04	56	+0.37	58	+0.40
	warm	51	+0.06	39	+0.06	51	+0.01	39	-0.01
Mean.			+0.092		+0.174		+0.159		+0.194

C ₂ lot.....	+0.120
C ₃ lot.....	+0.205
C ₄ lot.....	+0.100
Cold.....	+0.230
Warm.....	+0.054
Parents and offspring of same sex.....	+0.127
Parents and offspring of opposite sex.....	+0.158
Grand average.....	+0.142

but this holds true for each separate correlation measured, i.e., male parents and male offspring, male parents and female offspring, etc. The probable errors are, it is true, high, owing to the relatively small numbers of animals comprised.⁴⁴ But the coincident testimony of these four sets of figures indicates a considerable degree of probability.

Since we had independent evidence of a general tendency toward the reappearance of these parental modifications of tail length in the next generation, it could, indeed, have been predicted with considerable certainty that such a relation would be

⁴⁴ They are, in reality, higher than those given in table 24 (see foot-note to latter, pp. 417, 419).

found between these two sets of figures.⁴⁵ But it must here be insisted that these coefficients represent *correlations* merely, and not necessarily *heredity* in any restricted biological sense. As to the cause of these resemblances between parents and offspring, they throw no additional light whatever. The question is pertinent, however, whether the various "coefficients of heredity" which have been computed so frequently may not represent in part correlations in respect to somatic modifications, and not entirely correlations in respect to germinal differences, as is frequently assumed. This would be particularly likely in cases where the filial environment tended on the whole to agree with the parental, while the various parental environments displayed a considerable diversity among themselves. And does not such a condition hold true very largely for the human race?

A knowledge of this "coefficient of heredity" enables us to calculate roughly the degree of selection which it would be necessary to apply to a parent generation, in order that the mean of a given character in the offspring should be altered to a given extent. For example, we find from table 12 that in the C₄ lot the mean relative tail length of the warm-room section is greater than that of the cold-room section by 3.5 units (hundredths of the body length). Now, assuming a standard deviation of 5.0 for this character in the offspring (the mean of the 4 figures in the table), a standard deviation of 3.6 for the parents of either sex (this is actually known for the females only) and a correlation coefficient of 0.14, we find that in order to obtain a difference of 3.5 in the offspring, we must select two lots of fathers or mothers which differ by 17.9 units. Even if we allow a coefficient twice as great as that actually computed, and suppose that both fathers and mothers be selected, it is plain that the two groups of parents must still differ very widely in their mean (potential) tail length in order that we may obtain by selection alone such differences as were found between the two lots of C₄ offspring. The differences, indeed, would have to be greater than even the actual ones which were produced by temperature.

⁴⁵ To what degree, if any, the differences are due to the mere fact of greater heterogeneity of material," resulting from the method of computation (pp. 415, 417) I am unable to state.

GROWTH

In a former paper ('09), I called attention to the fact that the differences in tail length which were experimentally produced, as a result of temperature differences, diminished unmistakably during further growth, and that this diminution of the original differences occurred, *even when the environmental differences persisted in full force*. Additional data on this subject are presented in table 26. Here the absolute and

TABLE 26

Growth between first and second measurements; (comparison between cold and warm lots)

	TIME INTERVAL BETWEEN MEASURE- MENTS		NO.	WEIGHT		TAIL		FOOT		EAR
				Absol- ute in- crease	Rela- tive in- crease	Absol- ute in- crease	Rela- tive in- crease	Absol- ute in- crease	Rela- tive in- crease	Absol- ute in- crease
				gm.	per cent	mm.	per cent	mm.	per cent	mm.
B ₁ Super- fluuous males...	{ Jan. 5 to Mar. 17 }	cold	32	6.60	47.50 ± 2.68	9.34	15.12 ± 0.54	0.091	0.54 ± 0.16	1.089
		warm	52	6.52	47.58 ± 1.94	9.15	12.85 ± 0.43	0.129	0.76 ± 0.13	1.121
B ₁ Breed- ing males...	{ Jan. 5 to Aug. 22 }	cold	24	7.20	49.96 ± 3.12	17.75	28.08 ± 0.87	0.202	1.20 ± 0.19	1.432
		warm	29	9.14	64.76 ± 3.84	14.97	21.10 ± 0.59	0.163	0.93 ± 0.19	1.607
B ₁ females	{ Jan. 5 to end of Sept. }	cold	53	9.78	83.06 ± 3.00	19.49	32.08 ± 0.66			
		warm	56	9.84	74.83 ± 2.31	15.52	21.87 ± 0.42			

relative increase in respect to weight, tail length and foot length, and the absolute increase in ear length, are given for the periods which elapsed between two successive measurements of certain mice. By relative increase is meant the increase of the second figure over the first one, considered as a percentage of the first. Since the mean absolute values for the 'cold' and 'warm' groups differ somewhat, the relative figures are the more instructive.

Turning to the 'superfluous males,' it will be seen that the tails of the cold-room section underwent a relative increase of 15.12 per cent, as compared with 12.85 per cent for the warm-room section. That is, the more retarded parts have grown faster. Reference to figure 1 shows that during this period there was no diminution in the temperature differences. For foot length, the difference in rate of growth is slightly in favor of the warm-room animals, so far as is indicated by these averages. But it is to be noted that the amount of increase in either case has been insignificant, being in both instances considerably below 1 per cent. In fact, an examination of the individual cases (not here presented) shows that growth in foot length had very nearly ceased at the age of 50 days, the feet of certain animals actually seeming to show a slight shrinkage after that time. For ear length, likewise, the difference in the rate of growth is in favor of the warm-room animals, but here too this difference between the lots is very slight, and the absolute growth in either case is small.

For the next two lots of mice comprised in the table, it is again plain that the tails of the cold-room animals have grown faster during the interval between the measurements than those of the warm-room animals. The time intervals are considerably longer here than in the first case, including first, a period during which the temperature differences persisted undiminished, second a period of diminishing temperature differences, and third (after April 4) a period when the two sets of animals were kept under identical conditions. It is likely that most of the growth which occurred between the two measurements took place during the first of the periods named. For foot and ear measurements, the relations, as in the preceding case, are probably of no present significance.

The increase in weight, in the three lots, does not seem to have followed any intelligible rule. In one case, the two sections have increased at an equal rate; in another it is the warm-room animals which have grown faster; in the third, it is the cold-room animals.

While, therefore, no instructive results are evident from a consideration of the figures for weight, or the length of foot

and ear, we find conditions of decided significance in the case of tail length.

Unmistakable evidence has been given that the shorter tails of the cold-room animals grew considerably faster than the longer tails of the warm-room animals. The case of the first, at least, of these lots of mice is proof that this levelling down of the original differences occurred while the external cause of those differences persisted in full force.

Minot ('91) has shown for guinea-pigs that "any irregularity in the growth of an individual tends to be followed by an opposite compensating irregularity." This conclusion was based upon studies of body weight, and the irregularities referred to were not experimentally produced, but were familiar phenomena of variability. In the preceding paragraphs I have dealt with the levelling down of the artificially produced differences between the cold-room and warm-room animals of my stock. The question of differences among the animals of the same series has not thus far been considered. I am, however, able further to illustrate this principle of compensation by comparing the rate of growth in longer and shorter-tailed individuals exposed to the same conditions. The results are given in table 27,

TABLE 27

Growth in tail length between first and second measurements; (comparative rates of growth of longer and shorter tails in animals of each lot)

		ABSOLUTE INCREASE		RELATIVE INCREASE	
		Above average	Below average	Above average	Below average
		mm.	mm.	per cent	per cent
B ₁ superfluous males.....	cold	8.62	9.94	13.15	16.69
	warm	8.00	10.27	10.44	15.36
B ₁ breeding males.....	cold	17.33	18.00	26.00	31.43
	warm	13.75	16.14	18.17	23.93
B ₁ females.....	cold	17.76	20.89	26.81	32.70
	warm	14.63	16.71	19.53	24.92
Mean (116 above 110 below average).....		13.35	15.47	18.87	24.01

which, it will be seen, are in complete harmony with Minot's principle. In each of the six pairs of figures, that representing the growth of tails of less than average length is greater than that for tails of more than average length.

RELATIVE CONSTANCY OF RATIO BETWEEN TAIL LENGTH AND
BODY LENGTH

For reasons already stated, it was regarded as important to determine whether the tail length maintained the same ratio to the body length at all times of life, and in animals of all sizes. Table 28 gives the results of these determinations,

TABLE 28

To determine whether relative tail length (percentage of body length) varies with the size of the animal; comparison of mean for smaller and larger animals of each lot

	NO.	FIRST HALF (SMALLER)	SECOND HALF (LARGER)
A males.....	44	90.77	89.73
A females.....	116	92.90	92.76
B ₁ females, cold.....	52	83.77	84.12
B ₁ females, warm.....	56	89.39	88.04
B ₃ males, cold.....	56	93.96	92.96
B ₃ females, cold.....	44	96.86	92.36
B ₃ males, warm.....	18	95.22	94.22
B ₃ females, warm.....	30	96.73	94.13
C ₂ males, cold.....	44	92.18	91.77
C ₂ females, cold.....	50	94.16	92.12
C ₂ males, warm.....	30	98.00	93.93
C ₂ females, warm.....	32	97.94	95.06
C ₃ males, cold.....	42	88.76	88.90
C ₃ females, cold.....	52	89.88	90.73
C ₃ males, warm.....	46	87.43	87.61
C ₃ females, warm.....	40	88.65	88.45
C ₄ males, cold.....	56	90.18	89.21
C ₄ females, cold.....	58	90.72	90.24
C ₄ males, warm.....	50	91.00	93.52
C ₄ females, warm.....	40	97.45	92.40
1909 lot, males, cold.....	58	92.24	93.24
1909 lot, females, cold.....	52	94.00	95.19
1909 lot, males, warm.....	46	93.22	92.70
1909 lot, females, warm.....	38	96.42	96.00
Mean.....	1150	92.199	91.476

based upon 1150 animals. Each of the lots was divided into halves, one containing the smaller (shorter) animals, the other containing the larger ones. The mean relative tail length was determined for each of these sub-groups. Referring to the grand averages, it will be seen that the figure for the smaller animals is slightly greater (0.723) than that for the larger animals. Since this relation holds true in 17 out of 24 cases, it seems likely that it is not accidental. On the other hand, this difference amounts, on the average, to considerably less than 1 per cent. So that for the purposes of comparison, between groups of animals which agree closely in mean body length, this difference may be disregarded.

Whether or not the relative tail length differs at different periods in the life of the animal cannot be ascertained from my table. If such differences, due to age, actually exist they are of much smaller magnitude than the differences between different lots of the same age. At least, this is true for the period from 50 days to maturity.

SEXUAL DIFFERENCES

In tables 3, 4, 7, 8, 11 and 12 are given the mean differences between mice of the two sexes, computed according to the method of size-groups. It will be seen that in 10 cases out of 11 the sign of the difference for tail length is negative, i.e., the females have longer tails. The single exceptional (positive) figure is based upon a small number of individuals, and the difference shown is slight, compared with most of the negative ones.

In considering the meaning of the sexual difference in tail length, here shown, the method of measurement must be borne in mind. The tail length represented in these tables is the distance from the anus to the tip of that appendage. But in males the position of the anus is affected by the presence of the testicles, a fact which may be wholly responsible for the apparent differences in respect to tail length.

As regards foot length, the mean measurements for the male mice exceed those for the females in 6 cases out of 9, while the

three negative figures are all smaller than any of the positive ones. It is possible, therefore, that we have here a significant difference between the sexes in respect to this character, though the evidence is of course quite unsatisfactory.

The males are heavier in 7 cases out of 9, while for ear length the figures are about evenly divided.

The relations shown by the figures for tail, foot length and weight emphasize, however, the importance of treating the sexes separately in all such computations as those considered in the present paper.

SUMMARY

1. The present paper is a final report upon a series of experiments on white mice, which was commenced in the fall of 1906 and discontinued in the fall of 1911. In the course of these experiments about 2300 animals were subjected to measurement. The principal results of the experiments of 1906 to 1909 have already been published in a number of earlier papers. In the work of the last of these years, which forms the chief subject matter of the present report, about 1300 animals were measured.

2. The parent generation of mice were reared, usually from birth, in two rooms, differing from one another widely in temperature and relative humidity. During the experiments of the last year the mean temperature difference between these rooms was over 18°C. From first to last, about a thousand mice have been subjected to this treatment (including only those which lived to the date of measurement).

3. On four different occasions, a second ('F₁') generation was reared from parents which had been treated as above described, these F₁ animals being reared under conditions which were identical for the two groups of contrasted parentage. I have, altogether, measurements from 879 mice of this second generation, of which number 594 were reared during the last year of the experiments. It was not found practicable to secure an F₂ generation.

4. On one occasion a rather limited number were reared, born of parents which had been exposed for some months to these

temperature differences, but which were mature, or nearly so, at the beginning of the treatment. In this, and various other control experiments which were undertaken, I was seriously handicapped by the high mortality of the animals in prenatal and early postnatal life.

5. All of these various lots of mice were measured according to a uniform system, and the resulting figures have been subjected to rather extensive statistical treatment. The distinguishing feature of this treatment has been the grouping of individuals according to size (body length or weight) permitting of accurate comparisons between series of contrasted history or parentage.

6. Throughout these experiments, I have found certain differences between the mean measurements of lots which were reared in the cold-room and ones which were reared in the warm-room. As regards two parts, the tail and foot, these differences were considerable in amount, and of absolutely certain statistical significance. They were always in favor of the warm-room animals, and were evident in all of my four independent series of experiments. Comparison with the original stock shows that, in respect to tail length, at least, it was the cold-room animals which were chiefly modified, the warm-room animals probably representing more nearly the ancestral condition.

7. A similar modification of ear length, under the direct influence of temperature, seems to have been shown in one or more of my earlier series, but it is slight in amount and of inconstant occurrence. It was not shown with any probability during the experiment of the last year, although the lot then reared was larger than any of the previous ones.

8. During the experiments of two different years, comparatively small lots of cold-room and warm-room animals (in each case males) were killed before the withdrawal of the temperature differences, and compared in respect to density of pelage. In each case, the cold-room animals had an appreciably greater quantity of hair. These findings are based upon a consideration of 74 warm-room and 53 cold-room mice.

Some mature females (about 50 in each of the contrasted lots) which were killed nearly six months after the discontinuance of the temperature differences, showed a reversed condition, the warm-room animals now having more hair. In both cases, these differences are of very probable significance, statistically speaking, though they do not, of course, prove any *direct* effect of temperature upon the growth of the hair.

9. Temperature did not prove to have any constant effect upon body weight, either absolute or relative. During the experiments of the last year, the warm-room animals of the parent generation had a greater relative weight (i.e., when animals of the same body length were compared) than did the cold-room animals. But the reverse relation has been found in certain series, and no general rule can be recognized in this regard. In most of the series reared (including the largest) the mean body length of the warm-room lot was slightly greater.

10. In the offspring of these modified mice, born and reared in a common room, those of warm-room parentage had, on the average, a greater weight and greater length of tail, foot and ear than those of cold-room parentage, when animals of the same body length were compared. This conclusion is based upon four different lots, each containing approximately 200 mice. Of these four lots, three showed the relations stated, for both sexes and for each of the characters mentioned. The other lot offered a partial exception, the sign of the differences for tail and foot being reversed. These reversed differences were, however, smaller in amount than the others, and this single partial exception cannot outweigh the combined testimony of the other lots. As corroborative evidence, we may cite a comparison which was made between the offspring of modified parents and another lot which may, for the purpose, be regarded as a control (see paragraph 12).

11. In one of the four F_1 lots, born nearly 5 months after the discontinuance of the temperature differences, determinations of hair weight were made, with the result that the *warm-room* series was found to have the greater quantity of hair. This agrees with the later condition of the parents (at least

the mothers) at the end of the experiment, but does not accord with the earlier condition brought about under the immediate influence of differing temperatures.

12. In the limited series referred to in paragraph 4, born of parents which, after arrival at maturity, were exposed for several months to the differing temperature conditions, uncertain and contradictory differences were found between the animals of cold-room and warm-room parentage. This series afforded, however, an interesting basis of comparison with one derived from parents which had been kept from birth in the differing temperatures (see paragraph 10).

13. While the offspring of cold-room and warm-room mice have been found to differ from one another in various characteristic respects, provided that the parents were influenced by the temperature conditions from a very early age, it does not appear that these differences in the offspring are entirely parallel to those produced in the parents. It has been shown, for example, that the differences in weight and in ear length, while of constant occurrence in the second generation, are not present with any constancy in the generation immediately influenced by temperature. Indeed, as regards ear length, it is perhaps significant that the only clear case in which differences resulted between lots which were kept at differing temperatures, is one in which the preceding generation had also been subjected to these experimental conditions.

14. Figures are available from a very limited number of mice which were born of parents that had been kept in the cold-room during the first two weeks of their lives, and then transferred to the warm-room. An analysis of these figures points to the possibility that the effective action of temperature, in determining the character of the offspring, was exerted upon the parents during that early period of life when the temperature of the body varied with that of the atmosphere. If this evidence were sufficient to have much statistical weight, the case would be good in favor of a direct effect of the temperature, as such, upon the germ-cells. Temperature determinations, made later in life, render it very unlikely that the germ-

cells could be directly influenced by this agency after the first two or three weeks following birth.

15. However much significance we may concede to the facts referred to in the two preceding paragraphs, the possibility is not thereby excluded of a process capable of effecting permanent, and perhaps important, racial modifications.

16. One possible interpretation of the results which were earlier published by me has been definitely excluded in my later work. This is the chance that the temperature conditions, acting, as they did, on the female parent during pregnancy, may have determined at that time the modifications later found among the offspring. In the 1911 series, fertilization occurred in every case after the discontinuance of the temperature conditions, and in some cases several months after this.

17. Correlations were determined between all the characters which were measured. These were computed in two ways: (1) in general populations, containing individuals of very diverse size; and (2) within groups practically homogeneous in respect to size. The coefficients obtained in the first of these ways are comparable with the ones which have been more commonly obtained by students of biometry. They are naturally of much greater magnitude than those obtained by the second method. But the latter would seem to be of much greater biological interest, and it is of importance to note that, for the younger animals at least, all of these coefficients were found to be positive. In other words, even when animals of the same history and the same size are considered, individuals with longer tails have, as a rule, longer ears and feet, are heavier, and have more hair. The existence of such correlations doubtless accounts, in part at least, for the general coincidence in sign among the various differences which were found between the animals of cold-room and warm-room parentage. And it must be admitted that this fact likewise diminishes the significance of any detailed agreement which may be found between parents and offspring in respect to these differences.

18. As was implied in an earlier sentence, the younger and older animals differed in respect to the correlations found, the

latter showing a considerably lower degree of correlation. Indeed two of the coefficients (tail-and-weight and tail-and-ear) were negative for the older animals, when the correlations were based upon size-groups.

19. "Coefficients of heredity" (filial-parental correlation) were obtained for relative tail length (i.e., ratio of tail length to body length). Here, also, two different methods of computation were employed, and with differing results. In both cases, rather low positive coefficients were obtained.

20. Certain phenomena of growth were noted which are perhaps of some general importance. These are (1) that after the initial retardation of the cold-room animals in respect to tail length, the tails of the latter grew faster, both relatively and absolutely than those of the warm-room animals, even while the temperature differences remained in full force; and (2) that, in both lots, the shorter tails grew, on the average, faster than the longer ones. In either case we have a tendency toward compensation in growth, such as was long ago observed by Minot for the weight of guinea-pigs.

21. The ratio of tail length to body length was found to be slightly greater in smaller animals than in larger ones, when mice of the same age were compared. The difference was so small, however, as to be negligible for most purposes. No alteration of this ratio with increasing age was discovered, though it was not definitely disproved.

22. The only fairly certain difference between the sexes, in respect to the characters considered, related to tail length, the tails of the females, as measured by me, being slightly longer. This difference may be entirely dependent upon the shifting of the anus, due to the presence of the testicles, and may not concern the length of the tail proper. Weight and foot length appear to be less in the females, but these differences are not very certain statistically.

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ACCESSORY APPENDAGES AND OTHER ABNORMALITIES
PRODUCED IN AMPHIBIAN LARVAE
THROUGH THE ACTION OF
CENTRIFUGAL FORCE

ARTHUR M. BANTA AND ROSS AIKEN GORTNER

FOURTEEN FIGURES

EGGS OF *RANA SYLVATICA*

Treatment and general results

Last season (1913) some *Rana sylvatica* eggs, after having been centrifuged for another purpose, developed into larvae with extra tails or tail-like protuberances. The extra appendages were located, or projected, ventrally in various positions from the anal region to the throat. Such an accessory appendage occurred on a large percentage of individuals which developed to hatching.

In an effort to learn just what had produced this modification, eggs were again centrifuged during the present season. Unfortunately, we did not know at what stage the 1913 eggs had been centrifuged, at just what speed the centrifuge had been run, nor for how long the material had been treated. After considerable effort to find the correct stage of development at which to centrifuge the eggs and the correct length and vigor of treatment, similar modifications (and others) were produced again in the present season (1914).

Thinking the result possibly due to a transference of formative material just previous to the third cleavage of the egg (the first in the equatorial direction) we at first tried all stages from the unsegmented egg to four- and eight-cell stages, hoping by varying the treatments and running several sets of eggs at a time

to get the proper stage of development, vigor of treatment, and duration of treatment. We got no abnormalities of the sort expected. The eggs were either killed, failed to develop beyond the blastula, or developed into normal tad-poles. These results were apparent within three days after the first centrifuging was done. We then treated some later stages—late cleavage, etc.—along with the earlier stages in the next series of eggs centrifuged, and among the survivors found accessory tails in two individuals in one of the treated lots. These eggs had been subjected to a centrifugal force equal to about 180 times the equivalent of gravity for 10 minutes when in the early gastrula stage. This afforded a clue for further treatments. We then ran a double series, Series IX and X, of sixteen separate clutches of eggs. For comparison eggs in stages from the undivided egg to the late blastula were used and the treatments varied both as to speed and length of treatment. Six and in some cases seven lots of eggs of each clutch were treated at varying intervals. In all there were just one hundred lots treated. Since in this series no two lots of the same clutch of eggs were treated at the same time, in effect this constituted one hundred different experiments. This series of experiments gave typical results and no further discussion of other series need be entered into. The results were remarkably uniform, as table 1 shows.

In all the earlier stages, from the undivided egg to the 32-cell stage, a centrifugal force about 1700 times the equivalent of gravity for 2 minutes,¹ or 1350 times gravity for 1½ minutes, killed most of the eggs. The survivors failed to develop beyond the blastula. They are designated in the tables as ‘per-

¹ The centrifuge used was one of the electrical type of the International Instrument Company's make, having a radius of about 18 centimeters to the center of the egg mass in the tube. There were eight tubes so that eight lots could be treated simultaneously. According to the formula $F = \frac{mv^2}{r}$ (from Carhart's College Physics, p. 42) 2900 revolutions per minute produces a centrifugal force equal to about 1700 times the equivalent of gravity. Similarly, 2600 revolutions per minute equals about 1350 times gravity and 950 revolutions per minute equals about 180 times gravity.

TABLE 1

Showing summary of results of treatments in Series IX and X

Unsegmented eggs; 1st cleavage in progress; 2 cells; 2 to 4 cells; 4 cells; 4 to 8 cells	1700 times the equivalent of gravity for 2 minutes	Killed about 75 per cent; survivors mostly became 'permanent blastulas'
	1350 times gravity for 1½ min.	
	180 times gravity for 10 min.	
Between 8 cells and stage in which about 32 cells showed exteriorly	1700 times gravity for 2 min.	Killed all
	1350 times gravity for 1½ min.	
	180 times gravity for 10 min.	Killed many; others permanent blastulas. In two lots a few with spina bifida—in one of these lots many normals
32 cells to about 100 cells exteriorly	1700 times gravity for 2 min.	Killed more than half; others permanent blastulas
	1350 times gravity for 1½ min.	
	180 times gravity for 10 min.	Produced a few normals but nearly all had spina bifida or ceased development in the gastrula stage
About 100 cells to probably about 400 cells exteriorly	1700 times gravity for 2 min.	Killed all
	1350 times gravity for 1½ min.	
	180 times gravity for 10 min.	Killed many; others permanent blastulas Mostly with spina bifida, dying at an earlier or later stage of development
Later cleavage stages to time gastrulation probably just ready to begin	1700 times gravity for 2 min.	Killed all but some in one lot in which occurred some permanent blastulas, a few with persistent yolk plugs and one with spina bifida
	1350 times gravity for 1½ min.	Killed most; some normals; a single one with persistent yolk plug; many with accessory appendages
	180 times gravity for 10 min.	All normals

TABLE 1 (continued)

Blastopore evident or soon afterwards becoming evident	1700 times gravity for 2 min.	}	Killed most; accessory appendages in survivors
	1350 times gravity for 1½ min.		Accessory appendages in nearly all individuals
	180 times gravity for 10 min.	}	In those in which blastopore was not evident at time of treatment <i>all became normals</i> . Where blastopore evident nearly all developed accessory appendages
Advanced gastrula stages	1700 times gravity for 2 min.	}	Killed all
	1350 times gravity for 1½ min.		Killed nearly all; survivors normals
	180 times gravity for 10 min.	}	All normals

manent blastulas,² inasmuch as they developed to the blastula and remained alive as blastulas for as long as two or even three days, while the untreated eggs had meantime developed into larvae with the head and tail differentiated. One hundred and eighty times gravity for 10 minutes killed some, a great many became permanent blastulas, a very few developed spina bifida and some developed normally. From the 32-cell stage to a stage in which about 400 cells showed exteriorly, 1700 times gravity and 1350 times gravity for 2 minutes and 1½ minutes respectively, killed by far the greater part and the others became permanent blastulas; 180 times gravity for 10 minutes produced a few normals but nearly all became permanent blastulas or had spina bifida.

In the later cleavage stages, from the time about 400 cells show exteriorly until just before gastrulation begins, the higher centrifugal forces killed by far the greater part of the eggs; a few were normals, a few permanent blastulas, a very few had persistent yolk plug; one had spina bifida, and many had acces-

² The term 'permanent blastula' is not correct as applied to these embryos as a whole, for in some of them the lower hemisphere never took part in the cleavage and a true blastula was never formed. Hertwig ('99) has described and figured a large number of such embryos which he had produced by centrifuging.

sory appendages. These appendages were in almost all cases far forward on the head or throat region. All eggs treated at this stage with 180 times gravity for 10 minutes developed normally.

In a stage in which the blastopore was evident, or soon afterward became evident, 1700 times gravity for 2 minutes killed most individuals; the remainder developed accessory appendages. 1350 times gravity for $1\frac{1}{2}$ minutes killed very few and practically all individuals developed accessory tails. All individuals subjected to 180 times gravity for 10 minutes, when gastrulation was just ready to begin but in which the blastopore was not yet evident, developed normally. But those individuals in which the blastopore was evident at the time they were treated developed extra appendages when subjected to 180 times gravity for 10 minutes.

The accessory appendages produced in those treated just preceding or in the early gastrula stage were in many cases typical tails, though there also occurred in these lots cases in which the extra growths were horn-like protuberances on the head region. Some variations in this regard may be attributable to differences in stage or of development, for although clutches of eggs were never mixed we frequently noted some variations in developmental stages in a control, all of which were presumably of the same age.

Spina bifida

The types of spina bifida produced varied rather widely. In spina bifida the portions of tail anlagen coming from the two medullary folds cannot unite. When the proper developmental stage is reached each portion of anlage produces a tail-like structure. In many cases, however, the abnormality is so extreme that the embryo dies before that stage is reached. Such a case is shown in the flattened larva of figure 1, with the very broad yolk mass uppermost. The larva shown in figure 2 lived until a somewhat later stage was reached and tail-like protuberances began to develop. In other cases the medullary folds successfully closed except posteriorly and there were developed peculiar extensions of tail-like structures about the still

exposed yolk mass (figs. 3, 4). The tail-like structures are approximately symmetrical in many cases, especially in the earlier stages (figs. 2, 4) though in later development they are usually more or less twisted, due to unequal growth, so that, except for their bases, they may come to lie approximately in the median position. In other cases the structures are asymmetrical in origin. Not infrequently more than two tail-like projections are developed. Sometimes there are four, approximately symmetrically arranged—two postero-dorsal in position and two immediately ventral to them. In a few cases there were developed a normal tail and, ventral to the unclosed blastopore, two smaller tail-like projections. The tail anlagen had successfully fused in part but spina bifida was evident in the persistent yolk plug and in the two lateral tail-like structures ventral to it. The most interesting case of spina bifida found is shown in figure 5. In this individual a tail has developed normally, but ventral to the small unclosed blastopore another mid-ventral tail-like structure appears. Apparently the slight spina bifida permitted union of the anlagen both dorsal and ventral to a portion which persisted as an exposed yolk plug, which was also the position of the anus.

The fact that tail anlagen in this and other individuals were evidently situated ventral to the exposed yolk and that the anus later developed at this point, indicates a considerable amount of concrescence in this region of the developing wood-frog egg. For if development had proceeded normally this anlage should have joined the portion which formed the tail in the normal position and the anus should have appeared *ventral* to it. Smith ('14) has recently demonstrated considerable concrescence in this region in *Cryptobranchus*.

Accessory appendages not caused by spina bifida

To the writers' way of thinking, the extra tails, protuberances, etc., now to be described, are not related in point of causation of transference of material, to the accessory tail-like structure due to ordinary spina bifida. In cases of spina bifida the anlagen developed tail-like projections *in situ*.

The 'pseudo-tails' were developed as near to the normal situation for a tail as the mechanical difficulties of development in the particular case permitted. In the cases now to be described the extra growths were in almost any region *except* the anal region and did not occur in lots of eggs in which evident spina bifida occurred.³ In fact, there was a considerable gap between stages at which nearly all treatments produced spina bifida and stages in which the extra tails occurred. Within this gap only normal individuals occurred in the treated lots. Because of this fact the writers believe that the spina bifida and the abnormal tail structures accompanying this abnormality, and the accessory tails and protuberances produced at a distinctly and definitely known later stage of development, are not causally related. It would seem that the only explanation of these accessory tails, etc., as due to spina bifida, would imply that in the wood frog's eggs there is a stage during which susceptibility to spina bifida in centrifuged material is most marked, followed by a period in which there is no such susceptibility, and this in turn by another in which the susceptibility returns but manifests itself in a very different result. The facts do not seem to warrant such an assumption. Spina bifida was not noticed in the earlier stages of those lots in which the accessory tails, etc., later developed, yet all the lots were examined frequently. Furthermore, as previously noted, the tails and other protuberances were developed, not in the region of the yolk plug and anus as in the cases of spina bifida, but in almost every other position.

This matter has been considered at some length because figures 1 to 5, examined in connection with the other figures,

³ In one case a spina bifida and some persistent yolk plugs occurred, and in another lot a persistent yolk plug was noted in lots in which a few extra tails were developed, but this was most exceptional. While we had not examined the lots of eggs closely enough to state definitely that such was the case, we are convinced that these lots of eggs were in different stages of development when treated; that the most retarded ones were treated at a stage in which spina bifida or persistent yolk plug is produced; those most advanced in development were at a stage to acquire extra tails as the result of the treatment; while those in intermediate stages were not noticeably affected in their later development by the centrifuging.

readily suggest to the casual observer that the phenomena are all of one class and that some of the individuals are merely more evident cases of spina bifida than others.

The range of the occurrence of the extra tails has been sufficiently indicated. In many lots in which the extra protuberances occurred they were almost exclusively on the head region,—e.g., in one of our lots, 56 c, besides some normal ones, the following cases occurred: an individual with a blunt fleshy protuberance on the upper anterior part of the head; an individual with a rather slender protuberance somewhat enlarged at the end and extending ventrally from the antero-ventral head region; an individual with the yolk region much rounded and with an ear-like projection on the left upper yolk region just back of the head; another with a short broadly conical protuberance on the dorso-anterior region of the head; and a fifth with a blunt fleshy protuberance strictly frontal in position beneath which was a slender finger-like projection.

In other lots, besides similar protuberances, there were individuals with more pointed horn-like projections or fleshy curved projections in various positions on the head (figs. 6, 7). These projections and appendages of various sorts occurring on the head were in large part in eggs treated in a slightly earlier stage than those from which developed really tail-like accessory structures. In some cases, however, there occurred head projections and accessory tails in the same lot of eggs. Whether this was due to different developmental stages occurring in the same lot of eggs, cannot be stated with certainty, though the eggs in any given bunch often showed a considerable range in development.

There is no definite line of demarcation between the protuberances which occur on the head and are not tail-like in structure, and the typical keeled and *segmented* accessory tails in various locations, for all sorts of intermediate structures occur.

The typical accessory tails occurred, as already indicated, in those eggs treated in the late blastula and early gastrula stages. Figure 8 shows an earlier stage of development; figures

9 and 10 show the more fully developed structures. These originated almost without exception from the lateral muscle plate at the border between the yolk mass and the muscle plates and usually projected ventrally or ventrally and laterally and somewhat in a posterior direction. In structure they consisted of a more or less muscular part and a keel. The muscular portion shows the characteristic myomeres. The keel usually occurs only on the ventral edge of the tail, the dorsal edge being rounded and unkeeled; it has about the usual width in proportion to the size of the tail and is often much folded and crinkled, due to the curved or twisted course of the tail. In some cases, at least, the accessory tail had considerable power and freedom of motion and was moved independently of the normal tail.

In 55 f the following, among others with abnormal appendages, occurred: one with a segmented tail at the left side originating dorsally of the yolk mass three-fourths back from its anterior end and extending posteriorly and slightly ventrally; a second with an accessory appendage to the left of the mid-ventral on the anterior part of the yolk mass—a very blunt though flat-ended protuberance; a third with a large, broadly-keeled, segmented tail originating from an anterior ventro-lateral position on the yolk mass; and a fourth with a short but segmented and doubly-keeled tail originating from the left side near the posterior end of the yolk region.

The great amount of centrifuging which has previously been done with frogs' eggs has apparently been largely confined to material in the earlier stages, in which we failed to get abnormalities resembling the accessory appendages other than those obviously arising from spina bifida. As to the phenomenon of spina bifida, several different means for its production in the frog's egg have been found. Hertwig ('97, '99), Wetzel ('04), and others, mention its occurrence following centrifuging; Lillie and Knowlton ('98) and Schultze ('99) in eggs caused to develop at temperatures near zero; O. Hertwig ('94) likewise mentions its occurrence in eggs developing at low temperatures as well as in eggs developing above the optimum temperature;

Hertwig ('95 and earlier papers) and Morgan and Tsuda ('93) produced this abnormality by means of various salt solutions; and Hertwig ('92) observed the same type produced by polyspermy or overripeness of the egg at the time of fertilization. We do not know of the previous production of accessory tails and other appendages in Amphibian larvae.

Summary and conclusions

No material was prepared for sectioning and the writers can only offer suggestions as to the causes of the results produced. It is not believed that the accessory tails and the appendages produced on the throat and head regions of the centrifuged frog embryos were due to spina bifida, as many of the abnormalities which occurred in those treated in earlier stages obviously were. With two exceptions, no persistent yolk plugs or spina bifidas *and* accessory appendages—other than those obviously due to spina bifida—occurred in the same lots of eggs. In fact, there was a considerable interval of developmental stages between those stages in which centrifuging produced spina bifida and those in which centrifuging produced accessory tails and other appendages. In all cases in which there was any evidence of spina bifida the tail-like structures which occurred were in the immediate region of the exposed yolk—the tail anlagen developed tail-like structures *in situ*, when the developmental stage at which the tail is normally produced was reached. On the other hand the other accessory structures were produced in almost every region except that in which the blastopore occurred—in regions in which the occurrence of tail anlagen cannot be accounted for by spina bifida.

The writers suggest that the accessory tails which developed in various situations and the accessory appendages in the head region were due to the centrifugal force having transferred a portion (or portions) of the tail anlagen to the anterior region of the future embryo and that it there developed into a structure resembling a tail. On the head region it became merely a fleshy protuberance, but in connection with the lateral muscle plate it produced a tail more or less perfect in form, structure and function.

In the larva shown in figure 10 (upper figure) a double transference occurred, a portion of anlage developing in connection with each lateral muscle plate and two accessory tails resulted. Morgan ('06) sectioned and figured eggs of *Rana sylvatica* and *Bufo variabilis* in which a more or less local transference of egg substance had been produced by means of strong centrifugal force. This is a transference of material similar to that which we have supposed to have occurred in our embryos centrifuged in the late blastula and early gastrula stages.

Since these accessory structures were produced through the application of centrifugal force operating in the early gastrula stage or just preceding it, it seems reasonable to suppose that a portion or portions of the germ ring were detached and carried more or less toward the 'animal pole.' In the later readjustment of the various embryonic cells these anlagen became associated with various regions and developed into accessory tails or other projections, depending upon the region of the embryo with which a portion of the anlage became connected.

EGGS OF *AMBLYSTOMA PUNCTATUM*

Extensive series of eggs of *Amblystoma punctatum* were also centrifuged in an endeavor to learn if the accessory tail-like appendages might be produced in this form too. No such appendages occurred in any case, although many lots of eggs in all stages, from the unsegmented egg to the late gastrula, were centrifuged at several different rates of speed and for different periods of time. The nearest approach to an accessory tail or other appendages such as occurred in the centrifuged wood frog larvae was in the individual shown in figure 12. This projection from the mid-ventral region was apparently an epidermal sac containing largely a mass of yolk. Smaller—in most cases mammae-like—projections were noted on several salamanders of our series, including some in the checks. It is not believed the centrifuging had any relation either to the larger or the smaller projections on the yolk mass of the *Amblystoma* larvae.

Another salamander of the centrifuged material had two pairs of anterior legs. About 2 mm. posterior to the pair in the normal position occurred the second pair similar to it in size and position. The larva died and unfortunately was destroyed by Infusoria before its loss was discovered. The posterior leg buds had already appeared. The distance between the developing hind legs and the anterior pair of fore legs was apparently somewhat, though not much, greater than normal. The second pair of fore legs occurred about two-fifths the distance from the anterior fore legs to the hind legs. It is not clear how centrifuging could have caused this result and it seems doubtful if it had anything to do with it.

In several cases in the centrifuged salamander eggs, subjected to a centrifugal force 180 times the equivalent of gravity for 10 minutes, in the early gastrula stage, there occurred individuals in which the head development was incomplete (figs. 12 and 14). In many of these cases the larvae lacked the entire head. In a single lot (68 f) of eight larvae five possessed this abnormality. In one (fig. 14) the head rapidly narrowed off to a point almost immediately in front of the gills; the narrowed tip was near the level at which the eyes should have developed. In two others the head was even shorter and was cut off almost squarely. In one of these the two 'balancers' originated side by side almost in the median line and on the lower lip-like structure. (No true mouth or jaws were formed in these individuals with fore-shortened heads and in some of them there was no mouth opening at all). The head was about as rudimentary as the one shown in figure 12 but the tip was somewhat more prolonged. In a fifth the head was somewhat short; the eyes were imperfectly developed, though probably not functional. In the four previously described, the entire eye region was missing.

It had been observed in some of these centrifuged eggs that after removal from the centrifuge a blunt projection or exudate from the upper 'animal pole' constricted off and disintegrated. The writers did not observe whether these eggs produced the

virtually headless larvae, but inasmuch as in the urodele this is the region of the egg at which the anterior portion of the head is formed, the occurrence suggests that the material destined to form this portion of the embryo had been removed from the egg, thus virtually decapitating the embryo.

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PLATE 1

EXPLANATION OF FIGURES

Rana sylvatica embryos

- 1 Extreme spina bifida, resulting in a somewhat flattened larva with largely exposed yolk; further development impossible.
- 2 Extreme spina bifida; the tail anlagen began developing tail-like structures *in situ* before the embryo died.
- 3 Later stage of less extreme type; tail-like structures and exposed yolk mass conspicuous.
- 4 Embryo similar to figure 3, seen from a different angle.
- 5 Larva with sufficient spina bifida to prevent conerescence of the tail anlagen and cause the formation of two tails *in the blastopore region*.
- 6 Larvae with a protuberance on the head.

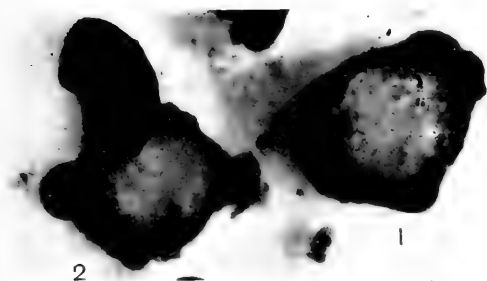


PLATE 2

EXPLANATION OF FIGURES

Rana sylvatica larvae

7 Larva with a fleshy, partly membranous projection in an anterior ventro-lateral position.

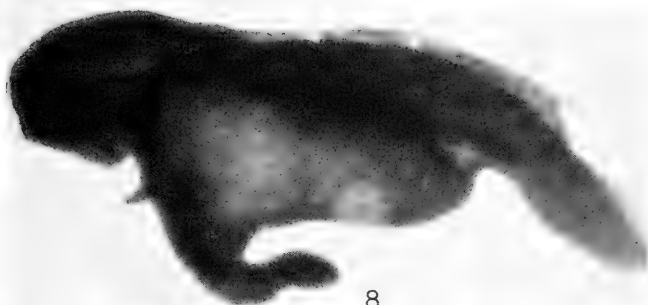
8 Embryo with a ventral projection arising from the lateral muscle plate on the left side and destined to become a tail.

9 Larva with a ventral tail (keeled on one side only) arising from the right lateral muscle plate.

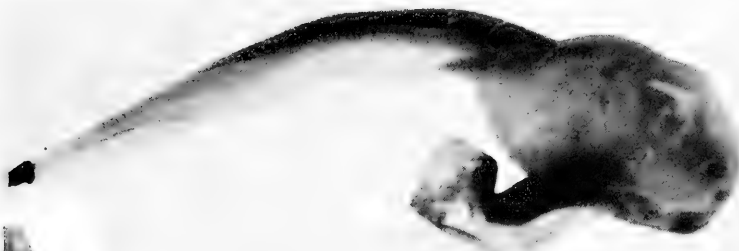
10 Larva (upper figure) with two accessory tails, one arising from each lateral muscle plate and projecting ventrally.



7



8



9



10

PLATE 3

EXPLANATION OF FIGURES

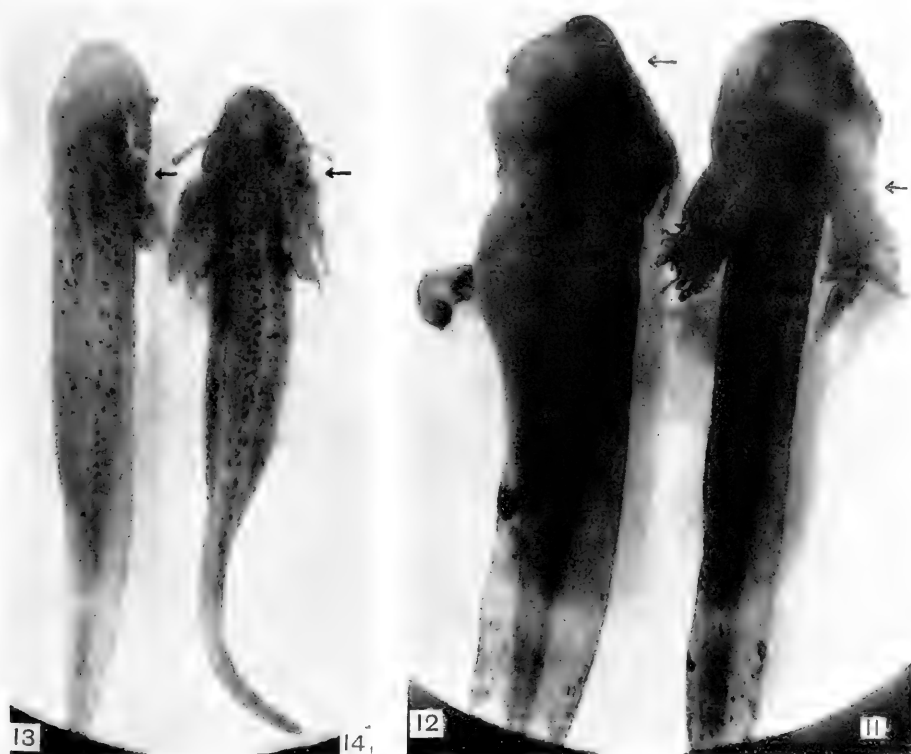
Amblystoma punctatum larvae

11 Normal *Amblystoma punctatum* larva.

12 Virtually headless larva, from a centrifuged egg of the same clutch as figure 11. The arrows beside figures 11 and 12 indicate corresponding levels on the two larvae.

13 Normal larva, younger than ones shown in figures 11 and 12.

14 Larva from the same clutch of eggs as figure 13 but from an egg centrifuged while in the early gastrula stage; while this larva has the head better developed than in figure 12 it lacks the eyes and other organs belonging to the anterior head region. The arrows indicate corresponding body levels in figures 13 and 14.



STUDIES ON INHERITANCE IN PIGEONS

II. A MICROSCOPICAL AND CHEMICAL STUDY OF THE FEATHER PIGMENTS¹

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SIXTY-THREE FIGURES—SEVEN PLATES

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¹ Papers from the Department of Experimental Breeding of the Wisconsin Agricultural Experiment Station, No. 4. The paper by Cole ('14) is the first in the series of "Studies on inheritance in pigeons."

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INTRODUCTION

1. *Method of genetics*

The aim of most of the work now being done on the study of inheritance is to identify in the hereditary complex the discrete units, factors, genes, which apparently control the external appearance of the organisms.

The method commonly in use to accomplish this genetic analysis is hybridization or cross-breeding. Forms differing in one or more respects are mated, the crossbred offspring are mated together and with the parents, and, depending on how the characters are distributed, the gametic structures of the animals are formulated.

The most that can be done by this method of crossing and hybridization is to discover the relation of one character to another as they *appear* in heredity, and from this we may form a hypothesis concerning the relations of the factors to each other as they *act* in the ontogeny of the organisms. We cannot get beyond hypothesis until we are able to go behind form and color as we see them, to the mechanical and chemical nature of the processes and the factors which control them.

Pernitzsch ('13) is attempting such an analysis of the races of Axolotl which Haecker has been using in his genetic experiments. After speaking of the hybridization methods as a means of identifying the factors of heredity, he says (p. 149):

Eine völlige Sicherheit ist wohl auf einem anderen Wege erreichbar, indem man nelmlich die Unterschiede zweier Rassen, die durch mendelnde äussere Merkmale verschieden sind, möglichst genau morphologisch und physiologisch untersucht, und ihre Erstehung und allmähliche Divergenz im Verlauf der Ontogenese bis zum Ei zurückverfolgt.

Pernitzsch, in his study up to the present time, has established certain striking differences in form and behavior of pigment-cells in the integument of the three varieties of Axolotl. Certain other simple analyses of this nature have already been accomplished and have illuminated the field and prepared for suggestive interpretation of the facts. As examples: The compound nature of the coloring in agouti hairs and the ability of this form to give rise to the other colors; the rôle of oxydases and chromogens in producing animal pigments; the inhibiting action of certain oxyphenols on this reaction and its bearing on dominant whites. The facts presented in this paper are offered as a contribution to the method for the experimental study of heredity, described above. The form used is the tumbler pigeon and the characters subjected to analysis are the various self-colors found in this variety.

2. Granular nature of melanin pigments

The pigments concerned in the production of color in *Columba livia* are of the class known as melanins. Melanin pigments, as far as is known, are granular in nature; often the granules are of definite shape and of measurable size. Post ('94, p. 491) states that a particular shape is peculiar to each genus of animals. For example, the pigment granules in the dog are whetstone-shaped; in the guinea-pig, short and thick; in pigeons, rods of measurable size. Haecker ('90, p. 70,) speaking of feathers in general, says that the pigment granules are oval, spindle-shaped or circular in form. He specifies that the granules of the rust-

brown feathers of a certain variety of pigeons are spherical and he gives some good figures of these. Strong ('02, p. 155) held that for birds the granule shape is always rod-like and of a definite size for each species. He later ('03, p. 269) qualified this statement to account for spherical granules which he discovered in the iridescent neck feathers from the pigeon. At this time he took the position, since abandoned by him, that these so-called metallic colors were dependent directly on the spherical form of the granules.

3. Origin of pigment in feathers

Investigations on the processes of pigmentation of the developing feathers have been carried on by Post ('94) working on the dove, Rabl ('94) on the chick, and Strong ('02), on the common tern (*Sterna hirundo*). Strong was fortunate in the choice of his material for this study, since in the tern the pigment cells are comparatively simple and the process is direct.

At least one fact concerning pigmentation in birds is now well established, that is, that the entire process is confined to the epidermal layer of the feather. Post and Rabl early agreed that this is the case, and Strong in 1902 laid considerable emphasis on the matter. A large number of feather germs has been examined in the course of my own work, and among all the preparations not a single case suggesting melanin formation in the pulp has appeared.

In the feathers of the common tern, which contain black pigment, Strong ('02, p. 165) described the pigment as first appearing in certain of the intermediate cells near the pulp region before the differentiation of the ridges. The pigment corpuscles, at first exceedingly small, increase in size, become deeper in color and more numerous, until they finally form a complete ball. These dense black balls, two or three of which are seen in each ridge, are the pigment-cells. When the pigment-cells reach this stage they send out processes which finally arrive at the future barbule cells, and in some way distribute pigment to them.

Although this description in its essential features will answer for the pigmentation of pigeon feathers, it must be considerably modified in details to fit the process in certain of the colors, as will be shown later.

Text figure A illustrates diagrammatically the parts of a fully developed definitive feather, while figure B shows the general relationship of the parts in the feather germ. It is believed these figures will facilitate following the discussion and understanding the positions and relations of the drawings in the plates. For an account of the structure of feathers the reader is referred to Mascha ('05), while the general process of development and of pigment formation is described by Strong ('02).

4. Materials and methods

As mentioned above, the material for this work was taken entirely from tumbler pigeons. These birds as they are handled by the fancier exist in six different so-called self-colors, namely: red, yellow, black, dun, blue and silver. Another self 'color,' white, due to the absence of pigment, is not considered in this paper. These six colors will be described in detail, as they are treated individually. The particular individuals mentioned in this work are among those used in the breeding experiments of the Department of Experimental Breeding at the University of Wisconsin. They are of known parentage and genetic constitution and can be identified in other publications of this department (Cole '12, '14) since the birds are referred to by their permanent record numbers. Young birds of any color were to be had almost throughout the year, so that, all things considered, the material available was almost ideal for an investigation of this sort.

The methods used by Strong ('02) were found to be generally satisfactory and were essentially followed. Portions of whole feathers were cleared in chloroform and mounted in gum dammar. The most instructive preparations for a study of pigmentation in the adult feather were made by shearing the barbules from the barb with a fine scissors, cutting parallel

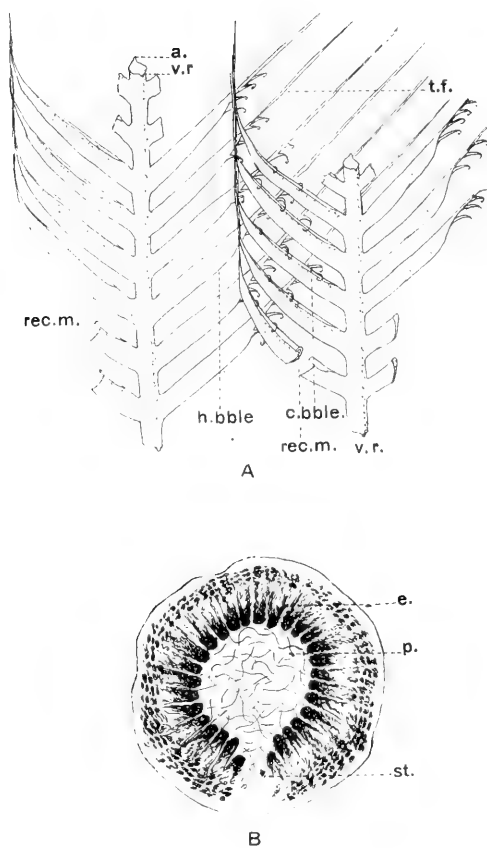


Fig. A Diagram of a portion of the vane of a cornified feather representing parts of two adjacent barbs with their two kinds of barbules, and showing the way in which the latter are interlocked by means of the minute hooks.

Fig. B Diagrammatic cross-section of a feather fundement showing the relations of the parts as they develop. At the lower margin is the shaft of the future feather, *st.*; the interior is filled with pulp, *p.*; the epidermal region, *e.*, is breaking up into ridges, each of which represents a barb with its barbules; the whole is surrounded by the feather sheath, represented by the single line.

with the barb and toward the shaft. In this way large numbers of barbules collect on the blades of the scissors and can be scraped on to a slide and mounted direct. This flattens out the barbules and exposes them to view throughout their whole extent. Sections of dry feathers were made by mounting in paraffin from chloroform and cutting on a Minot microtome. In order to obtain ribbons so as to preserve the parts in their proper relation on the slide, it was necessary to cover the cutting surface of the block with a thin film of celloidin after each section was cut.

As fixing reagents for feather fundamentals Kleinenberg's picro-sulphuric, and also the fluids of Bouin and Gilson, were found satisfactory. As a clearing agent xylol proved to be unsuitable, its action being to harden and to increase the brittleness of the feather germ. The use of chloroform obviated this difficulty. Best results were got when the change from chloroform to paraffin was made gradually, by saturating the clearing chloroform with paraffin before transferring to pure paraffin. For good results infiltration with paraffin was continued for at least three days. In sectioning, the series would ribbon off nicely near the umbilical end of the feather germ, but the sections showed an increasing tendency to fall out of the ribbon as the cornified part of the feather was approached. The final sections in the series invariably fell out of the ribbon, and it was necessary to pick them up carefully by a moistened needle, and remove them one by one to the slide.

In staining, for the most part, weak cytoplasmic stains were used, for it is easy to obscure the pigment granules, and to confuse them with other cell structures which stain heavily when any considerable coloration of the sections with basic stains is produced.

The invaluable suggestions and criticisms of Dr. L. J. Cole, given throughout the course of the work, have been an essential factor in accomplishing whatever results are here presented. The writer also wishes to express gratitude for valuable assistance given by Dr. R. M. Strong, at his laboratory in the University of Chicago, for a short period at the inception of the work.

EXPERIMENTAL FINDINGS

1. Red color of pigeons

a. Distribution of red pigment in the feather structures. The so-called 'red' of the domestic pigeon belongs to the series of browns rather than reds. Ridgway's ('12) chestnut (plate 2), or pecan-brown (plate 18), are nearest the typical red of pigeons. These colors may be bright or dull, light or dark, but the range of variability is small and reds of whatever kind are easily classified as such. The most extreme variance from the typical red is the color called, in our work, 'plum color,' or 'wine color,' to be considered later.

If the pigment masses in a preparation from a red feather are examined under a microscope by transmitted light they appear red-brown in color, very much as when examined by gross methods and reflected light. Apparently the individual granules in a red feather are never dense enough to exclude all the light, thereby appearing black by transmitted light. Further, it is impossible, in the small space within any feather structure, for the granules to be thickly and deeply enough packed to become opaque. When feathers have been cleared in chloroform and xylol even the thickest parts of the barbs are readily pervious to light and their red color unmistakable. Further evidence, if it were needed, for the existence of a pigment substance red in color, has been obtained by chemical methods, and will be brought out in the section devoted to that subject.

Typically, all the structural elements in the red feather contain pigment material. Figure 1 is a cross-section of a red feather vane, and shows this condition. If there is any reduction from the maximum pigmentation (i.e., light red) the first structures to suffer loss (the pigment-free areas at the cell boundaries are not considered here) are the haemules or hooks, followed by the hair-like terminal extension, first of the curved barbules and then of the hook barbules. In all kinds of red (also, as will be seen, in colored pigeon feathers generally) the hook barbules are more heavily pigmented than the curved. Figures 3 and 4 show the relative intensity of pigmentation in

these two kinds of barbules. Any reductions of pigment further than those in the haemules and terminal fibers, mentioned above, fall most heavily on the curved barbules; they can be almost devoid of pigment while at the same time the hook barbules show practically no reduction in pigmentation. This condition, which results in a grating of parallel pigmented and non-pigmented lines, is associated with the effect known as 'grizzled,' and will be dealt with more fully at another time.

The barb in typical reds contains some pigment (figs. 1 and 5). The cortex, in covert feathers, is uniformly pigmented from apex to ventral ridge. The cavities of the medullary cells are also copiously lined with pigment granules. In flight feathers, in which the barb is always thin and high the portions of the cortex beneath the barbules are more sparsely pigmented, causing these feathers to appear dull in color when viewed from the ventral aspect. Figure 45 is from a black feather, but shows this condition of distribution. The dull ashen color seen on the under side of the wing in typical red birds is due to this condition.

As pointed out, the region near the transverse walls of the barbule cells generally tends to be free of pigment. This serves to break up the pigment into separate masses, giving a segmented appearance to the barbule. The degree of this segmentation—that is, the breadth and clearness of this pigment-free area—has an influence on the color effect of the feather. Lightness and darkness in reds (shown later) depends chiefly on the number of granules per given area, but sparse pigmentation is generally associated with a broadening of the pigment-free band. The boundaries of this area are not cleancut, however. They are broken by invading rows of granules, and occasional granules may be found scattered within the area (fig. 4). When more granules are present the pigment encroaches on the pigment-free band, narrowing it and the red is darkened, or perhaps, more properly, enriched. Light and dark reds can be compared by examining figures 1 and 5 and also figures 6 and 7. Figures 1 and 7 are drawn from 5 B, a dark mahogany red; figure 5 is from 93 B, a 'washed-out' pale red, and figure

6 from a light red feather of 894 B. The light or dark condition is clearly a function of the number of pigment granules present and involves no change in kind or form of granules.

b. Granule form. In red birds the granule form is without exception spherical. The granules are for the most part, though not always, uniform in size in each barbule. The typical size is about $0.3\ \mu$. In typical reds, or even better in dark reds, where the spherical granules are packed in tightly, they assume more or less the arrangement taken by round shot if placed on a plain surface and crowded together, presenting the appearance of rows running with the length of the barbule, the granules alternating in the rows (fig. 7). This linear arrangement is not due alone to being packed into the cell, however, as it is also seen in the light reds where the granules are thinly scattered (fig. 6). Haecker ('90, p. 70) also observed this latter condition in referring to the spherical granules of the rust-brown pigeon feather, "welche in perlschnurartigen, der langsrichtung des betreffenden Organs folgenden Reihen angeordnet sind."

c. Plum color. There appears in some of our red birds, especially in the region of the breast and belly, but often spread over the shoulders and back, a greatly modified condition of the typical red. In our work we have spoken of this as plum color or wine color. Ridgway's indian red ('12, plate 27) is a near representation of this color. The color also lacks the brightness and warmth characteristic of red; it is cold and possesses a bloom like that seen on an unwiped plum rather than the sheen seen normally on reds.

The granules of pigment making up this color are spherical in shape but remarkably large in size, often reaching 2.5 to $3\ \mu$ in diameter (figs. 22-23). The distribution in the barb is much the same as in normal red, with the exception that the ventral part of the cortex bears fewer granules (fig. 24). In the barbules segmentation is always marked, and the boundaries of the pigment masses always clean-cut and distinct. These broad clean-cut pigment-free areas, combined with heavy pigmentation, doubtless play a rôle in producing this color effect.

d. Development of red pigment. First signs of pigment formation in red pigeon feathers appear in the intermediate and inner sheath cells some time before the formation of the ridges (fig. 8). The pigment appears promiscuously throughout this region as minute granular clumps, in or between the cells; at a somewhat later stage, but before ridge formation, this promiscuous elaboration of pigment becomes more general; the clumps are denser and more numerous. Often just before ridge formation this pigmentation is so generalized that one gets the impression that practically all the cells in the field are taking part in the process.

The epidermal layer is now cut up into the ridges, each ridge enclosing its share of pigment material and active cells, which are scattered more or less throughout the tissue of the ridge (fig. 10). At this time certain of the cells begin to increase in size and activity, pigment accumulates in them, becomes closely packed and either sends out its own branches or becomes associated with other branches and pigment paths formerly existing. These are the so-called melanoblasts, melanophores or pigment cells of writers. They most frequently lie near the base of the ridge, but have been found in all situations within the inner sheath cells.

The fact that such specialized cells do occur in developing feathers, that they are large, that they are actively forming pigment and that they extend apparently continuous branches into the outer regions, has led some workers in this field to ascribe the whole duty of pigment formation to them; some unusual substance is supposed to be resident in these cells which endows them alone with the faculty of elaborating the pigment, and further to distribute it to other cells of the tissue, generally by means of pseudopod-like ramifications. As Strong ('02, p. 168) pointed out, in the case of the dove, the pigment cell processes are irregular in form. In the tumbler pigeon this condition is extremely marked. In fact, the processes are so tortuous that in working with a series of cross-sections it is very difficult, often impossible, to establish their continuity. Bending as they must so sharply upon themselves, they appear in the

sections as isolated, irregular, elongated pigment masses, occurring promiscuously throughout the ridge tissue.

Klee ('86, p. 139) believed that besides the pigment from the branched cells there was another source; he says that "Anderseits sah ich an den Zellen der Strahlen, etc., noch eine sozusagen freie Pigmentbildung." Strong ('02, p. 170) has stated that he cannot deny that there is a free formation of pigment in the barbule cells independent of that supplied by the pigment cells. Winkler ('10, p. 618) observed in regenerating tissue of *Salamandra* "dass schon sehr frühzeitig die oberflächlichste kernlose Zellenlage reich mit Pigmentkörnchen erfüllt ist," and that these or other cells later developed into branched pigment cells. Pigment development in tumbler pigeon feathers furnishes demonstrative evidence that this faculty is by no means confined to the specialized pigment cells. As has been shown in the earlier stages, the process is exceedingly generalized throughout the tissue, and I am unable to conclude that this activity on the part of the intermediate cells in general, ceases upon the development of more specialized cells. Any of the intermediate cells, it seems, may be stimulated to increased pigment-forming activity, whatever their location, and all stages of activity are seen, from the extremely specialized branching cells to those forming but a few granules. It is even possible that the entire pigmentation of the feather may take place without the activity of specialized pigment cells. The series represented by figures 11 to 15 represents such a condition. Abundant pigmentation is taking place in this feather, but scarcely ever is a well-developed pigment cell seen. Figure 16 also shows the feather structures becoming well pigmented, but specialized pigment cells are absent.

2. Yellow

a. Nature and distribution. Among self-colored birds the colors red and yellow are perfectly distinct. Even the lightest reds and darkest yellows are easily placed in their proper class. In appearance these colors bear the relation to each other of dilute and intense conditions of the same pigment material.

That yellow is a dilute condition can be demonstrated by even a hasty microscopic examination of the feathers, and the fact is further borne out to the degree of certainty by a comparative chemical study of the two kinds of feathers. Microscopically the pigment is seen to be far more sparsely distributed than in reds. Often under strong light the pigment in the barbules is almost imperceptible. In cross-sections of cornified feathers, especially, the attenuated condition of the pigment is well demonstrated (fig. 17). As regards the relative distribution to the various feather parts, there are some constant differences from the conditions seen in red. In the barbules the segmented condition of the pigment is seldom to be seen (fig. 18) though the hook barbules may show it in a weakly developed condition (fig. 19). It is not likely that this condition is a fundamental one correlated with yellow color, since yellows are perfectly capable of developing this condition if enough pigment is present, as shown often in the hook barbules and even occasionally in the curved barbules in dark yellows. Also, as a further result of sparse pigmentation, the nuclear areas are not so well marked out. In all preparations of yellow that I have examined the hooks or haemules are always (unlike red) pigment-free (fig. 19).

The barb in yellow feathers (figs. 17 and 20) contains far less pigment proportionately than the barb in reds (figs. 1 and 5). In the former it is almost entirely confined to the apex; the lateral sheets of cortex and the medullary cells are usually pigment-free. Occasionally, however, large dense masses of pigment, irregular in shape but distinct in outline, occur in the cortex substance.

b. Granule form. The crucial difference between yellow and red, however, has not yet been mentioned. It is concerned with the nature and form of the pigment granules. As shown above, the pigment in reds exists as distinct clean-cut spherical granules, typically $0.3\ \mu$ in diameter. No such spheres have ever been found in a yellow feather, in the course of my work. The pigment exists as irregular formless clumps, or agglomerations. These blotches of pigment appear finely granular in nature,

but are so fine, that discrete particles are beyond the limits of a 1.8 mm. oil immersion objective (figs. 18-20). This difference between reds and yellows is sharp and is constant. When minute patches of reddish pigment are present on a dun, black or white feather, it is frequently difficult or impossible by gross examination to classify the color as red or yellow; but microscopic study of such areas serves unfailingly to distinguish the colors by virtue of the morphological difference above cited.

c. Development. As regards the development of the pigment in the yellow feather fundament, the process is practically identical with that described under red, except that the end-product is, on the one hand, distinct spheres, on the other, irregular pigment masses. A study of pigment formation in yellows bears out the assumption that a large part of the pigment is elaborated by non-differentiated intermediate cells. Specialized pigment cells often are not present at all in the basal regions, while abundant and generalized pigment formation is going on in the barbule region. Figure 21 shows a small, and rather inactive pigment cell (*pg.cl.*) such as is often seen in these feathers.

3. Black

a. Nature of the pigment. Haecker ('90, p. 70) assumes that the 'rust-brown' pigment, such as is found in red pigeon feathers, "underlies all brown, gray, black and blue colors," that is, outside of the structural distribution of pigment these colors bear only a quantitative relation to one another. These relations are hypothetically possible if the pigment substances were always seen by transmitted light, since in this case any particle of substance impervious to the visible spectrum would appear black, although by reflected light this particle might give any of the spectrum colors. I have found that a microscopic examination of black feathers at once reveals striking differences from red or yellow and immediately places them, on the basis of the color of pigment, in a wholly different category from these colors. This work has indicated that the color of the pigment in a black bird is a true black and not an effect produced by the massing of dark brown color material. In a black feather

regions can always be found where the pigment granules are thinly scattered so that individual particles can be examined. The smallest granules, about $0.3\ \mu$, are not altogether impervious to light. The light which they transmit, however, shows no effect of differential absorption and is essentially white. This gives a color to the granules which might be described as 'attenuated black' or dun. In those young regions of the feather germ where the pigment is so sparsely scattered and finely divided as to be perceptible only as a faint nebula, its identification as black pigment is already possible. Finally the existence of a black pigment substance is demonstrated beyond further doubt by its isolation and purification by chemical methods.

b. Distribution. Black feathers are richly supplied with pigment. Its distribution in the different feather structures is in general similar to that in red. As in red, the hook barbules are almost invariably more heavily pigmented than the curved barbules, and the pigment-free areas at the cell boundaries, though generally well marked, are frequently very narrow. An expression of the richness of the pigment supply in blacks is the condition of the haemules, which are here more heavily laden with pigment than in any other cases examined. In reds this region carries but slight amounts.

c. Granule form. As regards granule form, blacks present varying conditions. If the barbules are heavily pigmented and the granules closely massed, as is generally the case with hook barbules, the granule form cannot be determined. In the lighter colored curved barbules where the pigment is thinly scattered, regions can always be found where individual granules can be distinguished and their size and shape determined with fair accuracy.

A study of feathers from certain black birds in our colony shows the pigment to exist as well-defined, uniform, spherical granules about $0.5\ \mu$ in diameter, packed in the cell in much the same way as the granules in a red bird (figs. 27-28). If in such a bird feathers from various regions of the body are examined, this condition of the granules will be found throughout.

In those structures too heavily pigmented to allow the observation of individual granules, the uniformly 'pebbled' appearance of the surface of the pigment mass yet serves easily to distinguish it as made up of spheres; such a bird is 1037 B (figs. 27-28). Feathers from other black birds may show an entirely different condition, for example, 7 A. Practically no spherical granules can be found in this bird. The pigment in all parts of the body exists in rod form about 0.8 to 1.5μ long, and 0.2μ in diameter (figs. 25-26). In general, the long axis of these rods is parallel to the long axis of the barbule, though in the region of the old cell nucleus they are often tilted as if they rested or were pressed against the walls of the nucleus, thus forming in optical section, a roughly circular cavity. Cross-sections of black feathers show in all cases spherical granules, a result to be expected, for cut across and viewed on their ends, even the rod forms appear circular in outline. These birds, 1037 B and 7 A, are similar in color, in intensity, and in sheen.

The type of blacks represented by 1037 B, in which a rod-like granule is practically never seen, is not common. Neither do birds showing as nearly an absolute lack of spheres as 7 A appear frequently. The commonest condition is for one kind of granule to predominate greatly and the blacks can generally be classed as 'predominantly spheres' or 'predominantly rods.' A few cases have been seen where the rods and spheres were about equal in number. Sixty-five black birds have been thoroughly studied in this respect, but up to the present time the granule shape has not been found correlated with either age, sex, or genetic constitution. Conditions here are very suggestive and a more detailed study of this matter will be made in the immediate future.

d. Development. Black pigment, at its first appearance in the feather fundament, is not unlike red in form and behavior. Black pigment is seen first between certain of the outer intermediate and inner sheath cells, as a fine granular haze characteristic in color for the black pigment of the feather. The corpuscles making up this nebula rapidly increase in size, appar-

ently by a 'running together' of the finer particles, and well defined particles are seen at an early stage. Figure 29 shows the position in which this pigment first appears, and also the promiscuous way in which it is scattered throughout the tissue. The spot marked *X* is shown under higher magnification as figure 30. It is hard to see how this pigment could have arisen in any other manner than an autochthonous activity of non-differentiated intermediate cells. Meanwhile, the cells or regions elaborating this pigment become more numerous till a condition of general but very sparse pigmentation is produced. By the time ridges have formed, certain of the cells have increased in size and become specialized for pigment production. These pigment cells generally develop among the inner intermediate cells, but frequently they are found nearer to the future barbule cells. At a very early stage they send out branches which at first seem to have no particular direction (fig. 31) but later only those directed toward the barbule cells remain active; the others either become spent or are withdrawn.

The well developed pigment cells of black feathers furnish better evidence than do those of red, that the pigment processes are continuous and actually function as paths for the translocation of the pigment granules from the pigment cell to the outer regions of the barbule. Every pigment cell is provided with one or more such rays, and their course is not so devious as those in red feathers. In thin sections their turnings and sudden bendings have not been so frequently cut across as in the case with the much twisted ray of the red feather and their continuity is more easily established.

Of data which suggest the nature or manner of the force which regulates the granule shape in black, I have none whatever. Frequently in the feather germs of birds which bear both rod- and sphere-shaped granules, one pigment cell will be elaborating rods, while another immediately adjacent cell may be producing predominantly spheres; figures 32 and 33 represent two such adjacent cells.

4. *Dun*

a. General. Dun color in pigeons is produced by the same pigment which produces black; the difference between the two colors is chiefly a quantitative one. In duns the pigment is distributed in different feather structures in the same manner proportionately as it is in blacks, but of course always less in absolute amount.

Self-duns have some specific limitations, however, in regard to granule shape and size. The pigment is more finely, also probably more uniformly, subdivided than in black. The measurement of particles so minute as these granules is at best difficult, and with the apparatus which was available for this work, inaccurate. The granules of dun are plainly smaller than those of black—as near $0.2\ \mu$ as could be determined—and their perfect spherical shape is undoubted (fig. 35).

This lack of rods in self-dun birds is very striking, but even here it is not absolute, for at least two dun birds, 265 A and 892 B, display this form of granule. In these cases the admixture of rods is exceedingly slight, however, and one may safely say that self-duns are as a rule characterized by granules spherical in shape and less than $0.25\ \mu$ in diameter.

b. Dun color in other birds. The conditions described above are in all cases associated with self-dun color. We may have dun colors appearing in birds which are very unlike self-duns in genetic constitution, that is, in blue or in black birds, but in these cases the dun color is made up in a different way; a similar color effect is produced but a different set of conditions prevails.

The body or wing covert feathers from a blue bird are not uniformly colored from tip to base (fig. 46). The distal portion shows the characteristic blue effect, which gradually merges into a typical dun color in the proximal two-thirds (or thereabouts) of the feather. The granule form of the pigment in this portion of the feather is always rod-like. The rods are always longer than in the black birds; they are confined to the dorsal portions of the barbule, and they are not found in the

haemules. Such conditions are shown in figures 43 and 44 and will be dealt with later, under blue.

The color in black birds may become so weak in certain regions that it may be described as dun. This condition is found most frequently in the side-of-body contour feathers, or under-wing coverts near the axilla. This effect is simply the result of a quantitative reduction in the number of pigment granules, those which are present being characteristic in size and shape for black birds. The black is 'diluted' without the aid of the *dilution factor*. These four kinds of duns—namely, self-dun, dun on the bases of blue feathers, dun color in pale black feathers and the dun on the under side of black flight feathers—are similar to the eye, yet only one of them is the *character* known as dun in our breeding work.

Dun also occurs in the wing bars of a silver bird. Study of a microscopic preparation of such a dun could not distinguish it from the dun of a self-colored bird.

c. Development. The process of development of pigment in duns is essentially the same as that which has already been described for blacks. Figure 36 shows a young ridge with three pigment cells. The processes are unusually straight and regular. It will be noticed that one is advancing along the outer side of each lateral plate. This should be compared with the position of the pigment processes in blues where they are confined mostly to the axial region of the ridge.

5. Blue

a. Nature of blue color in birds. Haecker ('90), Krukenberg ('84), Gadow ('82) and others, showed that blue as seen in bird feathers is a 'structure color.' Franz Samuely ('11) discusses several cases of animal pigment substances, blue in color, occurring in the tissues of certain coelenterates, and in the integument of certain fishes. No blue pigment substance has ever been discovered in the integument of higher vertebrates, though this color is both brilliant and common, especially in birds. A thoroughly satisfactory explanation of the exact optical properties and relations of the feather structures which

produce blue color has not yet been given. This is a problem demanding elaborate and refined physical technique, and biologists have had difficulty in solving the problem.

The color called 'blue' in domestic pigeons has very little claim to that name. It is not at all comparable to the blue of the bluebird, jay or indigo bird, but resembles more the so-called blue of the rabbit or maltese of the cat. In other words, the color belongs more properly among the grays than among the blues. The 'gull-gray' of Ridgway ('12, plate 53) is a fair representation of the blue of the domesticated pigeon. Typical spectrum blue, however, is found among tropical members of the pigeon family.

b. Distribution of the pigment. The pigment in these feathers is the same as that concerned in the production of black and dun. In distribution and granule shape, however, it presents striking differences from these colors. It is, in fact, this peculiar distribution of the pigment in the tissue of the feathers which produces the blue effect. In the barb the pigment is restricted entirely to the apex (figs. 37-39). The lateral sheets of cortex are altogether without pigment and the medullary cells, richly supplied with pigment in black and in duns, are entirely free from pigment in blue feathers. This is in marked contrast to the conditions seen in the true blue shown by the birds above mentioned, i.e., the jay or indigo bird (Haecker '90; Strong '02). Here the medullary cells are packed with pigment which is overlaid with pigment-free cells and tissue.

Striking as are these conditions in the barb they are not the chief factors concerned in the production of the 'blue.' In the barbule the pigment, instead of being spread throughout the cells in a fairly uniform layer, is massed closely together in the middle of each barbule cell. Figure 40 shows part of a barbule laid flat on its side. This highly exaggerated segmental arrangement of the pigment provides large areas for the reflection of light, modified only by the properties of the keratin and uninfluenced by underlying pigment substance. Light from such areas would be substantially white. Now if these areas are finely interspersed with regions which reflect no

light (that is, black) ideal conditions for the production of gray are presented. The manner in which this gray is modified to give the blue effect will now be explained.

When in position on the barb the conditions of light reflections from the barbules are not so simple. On the feather the barbules are arranged as a series of thin, curved, lamellae set more or less on edge. The pigment masses are so situated within these lamellae that when viewed from either edge (that is, from either dorsal or ventral aspect of the feather) the pigment must be viewed through a certain depth of colorless semi-transparent keratin substance (fig. 37). Moreover, this pigment-free keratin presents to the eye a series of convex surfaces which offer other optical possibilities for reflection and refraction of light. It is the combination of these conditions which produces the condition called blue in pigeon feathers.

That the blue effect is produced by a layer of pigment-free material intervening between the eye and the pigment mass is further demonstrated by the condition seen in the base of blue wing coverts, and often even in black flight or tail feathers. It will be recalled that the wing coverts from a blue pigeon are not blue throughout their entire extent. Only the distal part is blue, while the proximal unexposed part is dun color. These color relations of the different regions of the feather are in evidence only when it is viewed from the dorsal (outer) aspect. Viewed on their ventral aspect these feathers are blue from tip to base. A study of figures 43 and 44 reveals the cause of these differences in color, seen on the two surfaces of such a feather. The clumping of the pigment is largely broken down, i.e., the pigment is spread, in the bases (proximal region) of these wing coverts, but the pigment in the barbule has spread in only one direction, that is, toward the dorsal, recurved, margin. The ventral half of the barbule is still pigment-free, and from this aspect the pigment mass must still be viewed through colorless keratin, producing the blue color.

Frequently the flight feathers of a black bird, which are a good black when viewed dorsally, show a bluish cast on their ventral surface. This is again due to the presence of practi-

cally pigment-free keratin, but in this case the keratin is of the barb, not the barbule. The high thin form of the barb in flight feathers will be recalled (fig. 45) and when the pigment is confined to the dorsal portion of this structure a bluish cast is produced on the ventral surface of the feather. This is more marked when the surface is viewed at an angle other than ninety degrees, than it is when viewed exactly at right angles, simply because more of the pigment-free keratin intercepts the eye. This condition on the ventral surface of the black flights is exactly comparable to the dullness or ashen color seen on the ventral surface of red flight feathers.

c. Granule form. The combination of factors which produces blue in a bird also has an influence on granule shape. The pigment in the blue regions of a feather, which occurs massed in clumps, invariably exists in spherical granules (figs. 40-42). The granules exceed in size the largest ever found occurring in a black feather. When massed in characteristic fashion they have a diameter of about 0.8 to 1 μ and are remarkably uniform and constant in this respect (fig. 42). If the pigment masses are small and the degree of compactness slight the granules assume a larger size, sometimes reaching 2 μ in diameter (fig. 41). The alternating rows and string-of-pearl-like arrangement of the granules are well marked in blues.

As pointed out in the discussion of dun, the pigment in the bases of blue feathers (which region is generally dun in color), assumes striking differences in form from the pigment in the blue region, that is, it invariably shows rod-shaped granules, larger in dimension and more distinctly marked than the rod granules of a black (fig. 43).

The pigment in the black bar of a blue bird consists of spherical granules, in this respect resembling black birds of the type of 1037 B (fig. 27).

d. Development. The study of the origin of the pigment in blue feathers is attended with certain difficulties and uncertainties. There are invariably on the common blue pigeon two wing bars of black color involving the secondaries and secondary coverts. These bars are made up, as shown in

figure 47, by a series of black spots on the exposed half of the vane. They do not always occur at the same level and they are not the same width in different birds. This condition excludes these feathers as material for the study of origin of the pigment in the blue because one can not be certain whether the sections are taken from the blue, or from the black bar portion of the feather. The 'wing bow'—that is, the region of the tertiary and lesser wing coverts—shows the so-called blue in its purest condition. It has been pointed out, however, that these feathers are not blue throughout their whole extent but blend into a dun in the proximal unexposed portion. Here, also the granules are rod-shaped, not spherical. These long slender rods always arise as such, while the spheres in the blue portion of the feather arise as spheres. In a study of the manner of origin of these two kinds of granules the stage of development of the feather germ and the level from which the series is cut must be carefully determined. The linear dimension of that part of the formative region where the origin of pigment is taking place is at best very small (about 1 mm.) and entirely different results are obtained, depending on whether the series is taken from a level which is in the distal blue region or in the proximal dun region or in an intermediate position. The stage of developmental advancement of the specimen studied will in turn determine from which of these levels the series must be taken. If the feather germ is old enough for the distal portion, which is blue (about two-thirds of the feather), to be even partially cornified, the series will necessarily be taken from the proximal dun part because the cornified region is too hard to section. No spherical granules will be seen at this level. A pigment cell from such a region is shown in figure 48; from the nuclear membrane to the end of the pigment process the constancy of this rod form of the granules is distinct and striking. In the mature feather this region will show a dun color.

On the other hand, if the feather germ selected is in a considerably earlier stage of development, the series will be taken from a level which is destined to be blue. Figure 49 represents such a region fairly well, but is near the border of the dun region.

Here the pigment is almost entirely in the form of spherical granules of a size characteristic for blue. In a feather slightly older than this, but younger than that mentioned in the preceding paragraph, sections through the growing portion strike an intermediate region where both rods and spheres are present (fig. 50). Moreover, in a series of sections from such an intermediate region those sections farther from the base show fewer and fewer rods (figure 49 is just within this region), while passing in the other direction the proportion of rods becomes greater and greater.

Without very thorough study these conditions might easily give rise to the opinion that in these birds the pigment arises as rods and is probably translocated as such, but as development proceeds the rods are metamorphosed into spheres. Strong ('03, p. 269), studying the spherical granules which he found in certain iridescent neck feathers of the pigeon, concluded that "in the ontogeny of these barbules they receive rod-shaped granules of melanin from typical pigment-cells or chromatophores During the differentiation of the barbule cells however these granules are metamorphosed from the rod-like shape to a spherical form." The possibility of explaining the origin of spherical granules in a red pigeon in this manner is quite out of the question, for at no stage in their development is anything like a rod-shaped pigment granule present in them. The facts presented in regard to the development of pigment in blacks and particularly blues are, in the mind of the writer, ample proof that in other colors of pigeons also pigment granules arise directly in the form which they will permanently hold and that the apparent metamorphosis of rods into spheres is to be explained by the peculiar relation of these forms described above.

Apparently, in the color under discussion there is less free pigment formation in the barbule cells than is the case with black or red feathers. Also, pigment translocation does not begin so early in blue as in black or red. Another condition which characterizes blue is the more straight, direct and orderly manner in which the processes of the pigment cells proceed

outward. Moreover, they are generally confined to the axial region of the ridge, whereas the paths followed by the pigment processes in other colors may extend in any direction and between any cells of the ridge. Clearly some force is at work which has a controlling influence in the transportation of the pigment. As a result of this influence pigment granules enter the barbule only on the inner (future dorsal) side, while in the other colors considered they may find their way into the barbule cells from all directions (figs. 31 and 11). Figure 51 is from a blue feather germ at a stage when the barbules are just beginning to differentiate. Only a part of the hook barbules are shown. The pigment here is scattered somewhat promiscuously throughout the barbules, but the amount of it on the outer (future ventral) side of the nucleus is always small. Figure 52 is a similar region at a later stage; the pigment has withdrawn entirely from the outer (future ventral) side of the barbule cells, but still occupies all of the inner side. In figure 53 we have a similar region at a still later stage. Here the pigment has finally become clumped in the manner characteristic for blue.

This series of figures is typical for all cases of developing blue; though the pigment is never spread throughout the barbule cells, as in the case of black, the extent of spreading in the early stages is considerably greater than in the completed feather; there is in fact a 'clumping process' which goes on in the feather, changing the conditions of distribution from what would produce a black (viewed from the dorsal surface alone, but blue on the ventral surface of the feather) to a completed blue.

Certain preparations from blue feather germs which I have among my slides present a striking variation in development. At the very earliest appearance of the pigment, conditions are the same as those commonly seen, but as pigmentation proceeds the granules assume an extraordinary size, sometimes three or four times that of ordinary blue. Figure 54 is drawn from a young region of a feather germ before ridge formation. It shows two centers of pigmentation in close proximity, in one of which the normal blue granule size is seen, while the un-

usually large granules are present in the other. Figure 55 is a pigment cell forming these granules shown in its position in the ridge. Five feather germs showing these conditions have been studied. The point of interest is that they have in every case been taken from the blue rump of an otherwise black bird. In this connection it should be mentioned that the rump color of a bird is apparently controlled by a factor different from that which produces blue on other regions of the body (Cole '14).

6. *Silver*

a. General. The condition known among pigeon fanciers as silver is a very pale smoky gray or pearl. The amount of pigment present in these feathers is exceedingly small, in fact it is with difficulty that any pigment granules at all can be discovered in some specimens. Microscopic examination of these feathers shows them to possess the distribution of pigment essential to the production of blue, that is, in the silver portions of the feather whatever pigment is present is clumped in the middle of the barbule cells (figs. 57-58). As in blue, the curved barbules generally show this clumping in the middle of the barbule cells to a less degree than do the hook barbules.

b. Granule shape. A study of the granule shape in silvers is made exceedingly unsatisfactory by the extreme roughness of the barbule surface (fig. 61). This roughness has been noticed in other birds by several writers and has been described variously as due to striae, ridges, knobs, pits, blisters or 'ghost granules,' on the barbule surface. They are scattered with great geometric disorder over the surface, and unquestionably make the feather a poorer reflecting surface than it otherwise would be. These irregularities, though apparently most abundant on silvers, are also evident on blues and often even on duns. As mentioned above, they are so marked on silvers that the exact boundaries of the granules are obscured and a knowledge of their size rendered uncertain (fig. 62). A study of the developing feathers, however, escapes this difficulty and furnishes evidence that in silver (fig. 59) the granule shape is similar to that of dun (fig. 35), but in size the granules are somewhat

smaller and their incomplete opacity in the microscopic field speaks for a lesser degree of density.

Silvers like blues commonly have wing bars and in the same position and made up in the same way. They are, however, dun in color instead of black and the conditions microscopically are typical of dun feathers. As concerns the development of silver, the process is the same as seen in blue and nothing further need be added.

7. *Chemical studies*

a. Methods. The obstacles encountered in a chemical study of animal pigments are of various and numerous kinds and the chemical work done on the pigments of the feather in this investigation was of a relatively superficial and general nature.

In general the feathers were treated much after the manner Gortner ('10 b) describes for sheep's wool. The weighed feathers are put into a beaker and a quantity of 2.5 per cent NaOH poured over them (20 cc. liquid for each gram of feathers). The feathers are then digested at boiling temperature till almost completely disintegrated—a period of about 25 minutes for black and 12 minutes for red feathers. The mass is then thrown on a porcelain filter lined with white silk cloth, and filtered by suction. Filtering is very slow and difficult on account of the gelatinous remains of the feather structures, particularly the shafts. The filtrate is now made slightly acid with HCl, which process throws down a very light flocculent precipitate. The precipitate is allowed to settle, is separated and washed several times by decantation, boiled in 95 per cent alcohol for a few minutes, which removes considerable of the keratin products, and is then dried in a weighed dish.

No claim is made for even approximate purity nor for exact identity of the material so isolated with the pigment as it exists in the feather structure. The work, however, has furnished some suggestive data, which the writer hopes will be considered only in that light.

b. Red pigment. When red feathers suspended in a 2.5 per cent NaOH solution are heated, almost at once the liquid becomes

colored. After a few minutes' digestion the solution has taken on the chestnut brown characteristic of the feathers and if the remaining feather mass be freed of all liquid by pressure it is seen to have lost most of its color. Thus the pigment goes easily and completely into solution, and after filtration the solution is a clear, transparent, red-brown. The precipitate thrown down upon acidification contains practically all the pigment, but the supernatant fluid nevertheless retains a brownish tinge. The pigment from yellow feathers behaves in exactly the same way.

c. Black pigment. Black feathers, in the first stages of digestion, impart no color whatever to the NaOH solution. Not until the barbules have completely broken down and the pigment granules themselves have been liberated, does the digestion mixture become black in color. The black granules are extremely resistant and even after long digestion microscopic examination of a drop of the mixture reveals many of the granules intact in their original size and shape. The filtrate, as it was obtained, contains considerable pigment yet in granular form, but also much in a dissolved condition. Acidification throws down practically all the pigment material and the supernatant liquid is practically clear.

The red pigment dries slowly, remaining for some time as a pasty mass which becomes darker as it dries. When completely dried and powdered it is a very dark brown, but distinctly different from black pigment in the same condition. The black pigment dries without difficulty and without apparent change.

d. Quantitative relations. An attempt was made to determine the quantitative relations between red and yellow color, and between black and dun. The figures presented in table 1 do not represent absolute amounts of pigment in the feathers. As the pigment was weighed it contained keratin degradation products and salts left in by incomplete washing. The feather masses were all treated by the same methods, however, and the figures do approximate the truth as regards the relative amounts of pigment existing in the different kinds of feathers.

TABLE 1

Showing the relation of pigment content in dilute and intense birds

COLOR OF FEATHERS	WT. FEATHERS USED; GRAMS	WT. PIGMENT SECURED; GRAMS	PER CENT OF PIGMENT	RATIO OF INTENSE TO DILUTE
Red.....	4.415	0.2	4.530	
	10.270	0.385	3.747	
			av. 4.138	
Yellow.....	4.435	0.055	1.26	3.28:1
Black.....	7.80	0.26	3.333	
Dun.....	7.55	0.09	1.191	
	3.80	0.04	1.053	
			av. 1.122	2.9 : 1

The data in table 1, crude as they are, give us an idea of the magnitude of the influence of the factor for intensity, and furnish a numeral expression for its value. Acting in the skin of either a red or a black bird it appears to increase the amount of pigment laid down by about three times over what it would have been if this factor were lacking. Sufficient material for a study of the quantitative relations of pigment in blue and silver feathers was not available at the time, and these determinations were not made.

Much time and labor were spent in the course of this work in an attempt to demonstrate, in the soft growing regions of the feather tissue where pigmentation is actively going on, the presence of any oxydases or enzymes which might be concerned in the process. Young birds, fifteen to twenty days old, were killed, remeges and rectrices pulled out, the soft unpigmented portion cut off and placed in a mortar. This tissue was triturated with sand and extracts were made with distilled water, normal saline solution, chloroform water, and glycerine. Varying quantities of these extracts were added to tubes containing solutions of different amino acids, chiefly tyrosin, the reaction carefully controlled, H_2O_2 added to certain of the tubes and the tubes incubated.

The presence of tyrosinase, an oxidase which is a true enzyme, in the body wall of various forms, has been repeatedly demonstrated by several workers, particularly Gortner ('10 and '11) for insects; and Durham ('04) has reported the occurrence of tyrosinase in the skins of fetal rabbits.

In the writer's own work, as outlined above, many cases of pigmentation *in vitro* were obtained. The colors produced, however, did not correspond with the colors of the birds from which the extract was taken and their behavior with solvents and reagents was entirely different from that of the pigments prepared from adult feathers. Despite the best efforts evidence of bacterial action was often detected, which action alone might account for the appearance of color; and moreover repeated attempts often failed to reproduce cases of pigmentation once secured. Plainly this phase of the work warrants no conclusions as to the biochemical nature of the process concerned in pigmentation.

Summary of facts

The following points have been brought out in the preceding pages:

1. There is a red-brown pigment substance which produces the colors red and yellow in tumbler pigeons.
2. In red birds this pigment always exists as spherical granules, which are in 'typical red' about $0.3\ \mu$ in diameter, but in 'plum color' they are $2.0\ \mu$ or more in diameter.
3. In reds abundant pigmentation takes place in the intermediate cells of the epidermis, independently of specialized pigment cells.
4. In yellows the pigment is so finely divided that its granule form can not be determined.
5. There is a black pigment substance which under different conditions produces the colors, black, dun, blue and silver.
6. In black birds this pigment may exist as spheres $0.5\ \mu$ in diameter, or as rods $1.0\ \mu$ long. Some blacks show entirely one category of granule form, and some entirely the other. In most blacks, however, both kinds are to be seen, although there is commonly a marked predominance of one or the other type.

7. In blacks the pigment cells are better developed than in reds and elaborate a greater proportion of the pigment.

8. Pigment granules in dun birds are invariably spherical in form and about $0.3\ \mu$ in diameter.

9. Dun color may be seen in birds which are not dun genetically, but differences in granule form and distribution show that this is not the character dun which behaves as a unit in inheritance.

10. 'Blue' of pigeons is produced by the black pigment clumped and distributed in such a way as to produce the blue effect.

11. Granules in blues are spherical, $0.8\ \mu$ to $1.0\ \mu$ in diameter.

12. The development of the pigment in blues is not so promiscuous and 'disorderly' as in blacks. It is apparently controlled by an influence lacking in blacks.

13. After the pigment arrives in the barbules it undergoes a 'clumping' process which gives it its characteristic distribution.

14. The feathers from blue rumps on black birds show granules much larger in size than those in other blue feathers.

15. Pigment in silver feathers is distributed in the manner characteristic for blue.

16. In silvers the barbule surface appears greatly pitted and roughened.

17. The red and the black pigments show pronounced differences in their behavior towards reagents and solvents.

18. The intensity factor has about the same quantitative value in blacks and reds, and results in the formation, in intense birds, of about three times the amount of pigment present in the dilute birds.

DISCUSSION

1. The Mendelian factors concerned

It is interesting now to consider the bearing which the facts presented have on our interpretation of color inheritance in pigeons and on the pertinent questions of genetics generally. Cole ('14) in his breeding work with pigeons, has postulated certain genetic factors which are concerned in the formation of the six self colors (excluding white) of tumbler pigeons recognized

by fanciers. The ultimate recessive of these six colors is that known as yellow. A reddish pigment is seen in birds of this constitution and to account for its existence we may conveniently assume a factor *R* for red pigment. This red factor is apparently a fundamental one and no birds have been found which do not carry it. Strictly speaking, this factor is not entitled to a name until it has been seen to drop out of the hereditary complex and has later been re-introduced in an unchanged condition. But it is desirable to have something to which we may refer when occasion demands, and remembering the possible compound nature of the factor—in fact, that *R* might stand for ‘residuum’ just as well as for red—utility will be served by using the term.

There is a factor *B* for black pigment, dominant or epistatic to *R*, which added to a yellow produces black pigment and, when not acted on by other modifying factors, results in a silver bird. Another factor—*S* for ‘spreading’—added to a silver bird produces the condition known as dun. Moreover, these three conditions can be modified by the action of an intensifying factor *I*, which changes the above colors into red, blue, and black, respectively. Thus the six self colors and their genetic relationships are accounted for.

2. Influence of the several factors

a. Influence of factor B. When the factor *B* is introduced into a red or a yellow bird changes are brought about in the processes that go on within the feather fundamēt, which result in black pigment being laid down instead of red. The results of Bertrand ('08), von Furth and Schneider ('01) and many others, have shown that oxidases, acting on certain amino acids as chromogens, are able to produce a whole series of color changes. Riddle ('09, p. 329) in discussing the factor hypothesis for color, assumes that “in an animal that produces melanic color there exists all the machinery necessary to produce a series or scale of these colors,” and that the different colors seen in animals represent simply degrees of stages in a single process.

The black pigment in pigeon feathers by virtue of its color and its resistance to stains can be detected and studied at a very early stage in development. Lower down in the feather fundament, in the exceedingly finely divided stage when the pigment first becomes visible under a magnification of 1200 diameters, the fine haze is easily seen to be distinctly black in color. The possibility cannot be denied that at a time when the quantity of pigment was so small and its subdivision so fine as to be altogether invisible, the process of pigment formation might already have passed through a red stage. However, in higher and more developed regions of the feather where the rate of pigmentation is tremendously increased new pigment masses are forming. The process here is so rapid that even in their earliest stages pigment masses are sufficiently dense to be distinctly seen, and there is no indication of a red tinge in these masses at any stage.

Another fact which speaks for the complete developmental independence of these two pigments is their behavior physiologically. As pointed out (page 469) 'free' pigment formation is much more abundant and generalized in reds than in blacks. Oftentimes large, specialized pigment cells are rare in the former, while practically all the pigment comes from these cells in black feathers. This is a difference which might be overlooked in a hasty study, but examination of a large number of series substantiates the conclusion with compelling force. There is little ground for the assumption that merely an activation of the pigment-forming mechanism to a further stage should thus change the place of origin and manner of distribution of the pigment. When the factor *B* is added to the constitution of a yellow bird (*Rbi*) there is also a striking morphological change in the pigment, the irregular, formless masses of yellow being substituted in the resulting duns or silvers for spherical granules $0.3\ \mu$ in diameter.

We can thus study the properties of factor *B* by observing its influence in several distinct and independent ways on the pigmentation processes of the feather. The changes which it produces in the color, the mechanics of formation and distribution,

the physical form, and the chemical properties of the feather pigment all speak for an individuality and a value for factor *B* coördinate with *R*, *I* or *S*.

b. Factors concerned in the production of blue. The nature of the blue color, and the processes concerned in its production have already been discussed (p. 471). Though we are hardly justified in the statement that blues pass through a black stage, yet it is clear that black is a less differentiated condition of pigmentation than is blue, and if translocation of pigment should be arrested in a blue feather at the proper stage the resulting color would be more nearly black than blue (p. 477, fig. 51). This study furnishes strong evidence that some controlling force (we might call it a factor *C*, for 'clumping' is at work from the very beginning of pigmentation, which carries development beyond this non-differentiated point. It guides the pigment branches in their course, assembles the pigment into clumps, regulates the size of granules, and results in a blue feather. The assumption of a positive factor *C* for clumping in blue birds lead us, however, into logical difficulties, for blue is recessive to black, in which clumping does not exist. The pigment in blacks in fact, is spread uniformly throughout the barbule and this property (i.e., the spread condition of the pigment) was chosen by Cole ('14, p. 325) as a vehicle for a symbol representing a positive character present in the dominant blacks, and the factor *S* for 'spreading' is used by him. But although the factor *S* for spreading satisfactorily indicates the conditions in the adult feather, and although it makes good workable formulae and accurately expresses genetic relationships as revealed by experimental breeding, yet it is not compatible with developmental processes as they are unmistakably revealed by a microscopic study. On the assumption of a spreading factor, when a black ♂ (*BIS*) is mated to a blue ♀ (*BIs*) there is introduced into the 'blue' egg an element *S*, the action of which in ontogeny is to disperse the clumps, spread the pigment uniformly throughout the barbule cells, reduce the size of the granules—in fact, to produce a black bird. But plainly, black birds are not the result of such a series of changes. *S*

does not disperse the clumps, for they are never allowed to assemble in its presence; it is not S which is accountable for the spread condition of the pigment in blacks, for this condition exists, in certain stages of development, in birds which lack S . S , then, instead of being a factor which initiates developmental processes in ontogeny is rather a factor which prevents them from taking place; under the influence of this factor the processes which would take place in the primitive blue are stopped at a state short of completion, i.e., at a stage when conditions of pigmentation are such as to cause a black bird. It seems wise to the writer to retain the symbol S in the genetic formulae for pigeons, but with this modification, that we consider it a factor for 'stopping' rather than for 'spreading.' In the absence of the inhibiting influence of S (i.e., s) ontogeny runs its primitive course and produces a clumped condition: a blue bird. With this understanding of the factor S the logical relation between the positive and negative characters are maintained and yet a nice adjustment of hypothesis to fact is secured.

It must be recalled (Cole '14, p. 326) that the existence of factor S can be seen only in birds which carry B , for in birds lacking B (red or yellow) no conditions of coloration have been found to be correlated with the presence or absence of S . S may or may not be present in birds lacking B , but the alternative must be decided by a breeding test. In other words, S appears to act only on B and not upon R .

c. The influence of factor I . The most obvious and without doubt the essential influence of factor I is to stimulate the pigmentation process so that the amount of pigment laid down in intense birds is about three times the amount found in dilute birds. In this respect, I probably has a constant and uniform effect, whether acting on a yellow, silver or dun bird.

But besides producing this quantitative change, the factor for intensity acts in another totally unrelated way by regulating the form of the particles in which the pigment exists. However, I produces by no means a simple and constant change on granule shape when acting on dilute birds of different constitution, i.e., yellow, silver or dun. The irregular ill-defined

pigment masses of yellow feathers are substituted in reds by clean-cut, optically perfect, spherical granules. These two conditions are beautifully differentiated; there is never the faintest tendency for spherical granules of red to appear in a bird lacking the *I* factor, and vice versa. In dun birds *B* alone, without the aid of *I*, insures the existence of the pigment in spherical granules, again $0.3\ \mu$ in diameter. In other words, the secondary function of *I* as judged by its action on yellows has already been fulfilled by *B*. *I* has a further influence in a dun (*RbIS*) bird, however, and the size of the spherical granules is increased from 0.3 to $0.5\ \mu$; also an entirely new form, rod-shaped granules may appear. Of course one could with equal justice argue that these latter changes are produced by *B* alone when acting on a red (*RbI*) bird, and strictly speaking we should say that *I* acting with *R* alone has a different effect than when acting with both *R* and *I*. When *I* is added to silver (*RBis*) the $0.3\ \mu$ spheres are again increased, in this case from 0.8 to $1.0\ \mu$ in diameter; and moreover rods, longer and more slender, appear in certain regions.

TABLE 2

Showing the changes in granule condition brought about by the factor for intensity, I, when added to birds of dilute constitution

DILUTE			
GENETIC FORMULA	COLOR	GRANULE CONDITION	
		Shape	Size
<i>RbIS</i> (or <i>s</i>).....	yellow	undeterminable	undeterminable
<i>RBis</i>	silver	spheres	0.3μ
<i>RBIS</i>	dun	spheres	0.3μ
INTENSE			
GENETIC FORMULA	COLOR	GRANULE CONDITION	
		Shape	Size
<i>RbIS</i> (or <i>s</i>).....	red	spheres	0.3μ
<i>RBIs</i>	blue	spheres and rods	$0.8\mu-1.0\mu$
<i>RBIS</i>	black	predominatingly spheres or rods	0.5μ

Table 2 summarizes the changes in granule condition when *I* is added to birds of dilute constitution.

This method of studying the properties of a factor by observing its effects when acting in combination with other factors, is the best method available to genetists, but it is a superficial one. As an analogy, let us assume that we are to make a study into the chemical properties of a given substance and that the investigation must be prosecuted by the use of methods similar to those above. A series of beakers is obtained containing substances and mixtures of substances which happen to be at hand; the true chemical natures of these substances and mixtures are themselves unknown. The substance under investigation is introduced into the various beakers and the resulting changes described on the basis of color, granular or flocculent nature of the precipitate, the shape and size of crystals, etc. Clearly, with this information alone, we are a long way from understanding the absolute nature of the substance in question, but we have at least amassed a certain amount of definite data, such as it is, concerning it: we have done the best we can under the circumstances. It is largely to such empirical and superficial methods that the modern genetist is limited in his attack on the nature and properties of factors.

3. Granule shape in blacks

Excluding blacks, the wide and inconstant differences in granule form above discussed are associated with the presence or dropping out of what is otherwise a single unit factor, *I* (intensity). The case of the granule form in blacks must be developed further. Unlike other groups, the granule form in these birds bears apparently no simple constant relation to their color, black. To be sure, blacks of whatever sort are characterized by certain features which distinguish them from duns or blues, but within the color there are distinct types, and the distribution of these types is apparently without regard to genetic constitution, as it has yet been worked out, neither does it bear any relation to age or sex. That the granule shape may be here controlled by a separate factor which has this specific effect,

would be the easiest explanation. But it must be assumed further than the factor is powerless to exert any influence unless acting in a bird of black constitution, for as mentioned above, all the other colors are invariably characterized by their own peculiar conditions of granule form. This, of course, makes possible numerous genotypes of other colors, which carry the factor for granule shape as a cryptomere. A research to test this hypothesis is now in progress.

4. Analysis of characters

This study of granule shape amounts, in effect, to a partial and preliminary analysis of certain unit characters of pigeons. We are now able to speak of them in terms of their anatomical and morphological nature, rather than on the basis of general optical effect. We have been wont to describe these color characters by a single term, and have been assuming them to be as simple as our description. However, the facts brought out by this study are highly suggestive that underneath what we can see macroscopically and what we name, a whole series of changes is being enacted; there is an elaborate interaction of factors moulding the size and form of the pigment granules, creating, in effect, new characters as distinctly different from each other as blue color is from black, as seen by the naked eye. It is improbable that these various conditions of granule form are in any way optically concerned in producing the colors with which they are associated. The base of a blue feather contains long slender rods of pigment but is just as good dun as the feathers of a self-colored dun bird; and a lightly pigmented side-of-body contour feather near the axilla, from a black bird, can not be distinguished by color alone from a true dun, though the spherical granules are one-third larger and the pigment may even exist entirely as rods (p. 470). Likewise blacks (p. 468) identical in appearance in every way may contain granules of entirely different categories.

It is natural that geneticists should first have been entirely occupied with the obvious characters. When we limit ourselves to such characters, however, we are working the surface of the

field only; failure to see it with the naked eye is no assurance that a microscopic mechanism is not at work, and it is easily possible that previously baffling ratios may be explained by the revelations of a more minute analysis.

5. *Concluding summary*

A "Summary of facts" containing a brief statement of the experimental findings has already been given (p. 482). The following summary is concerned with the "Discussion" (pp. 483-491) only.

1. The six fundamental self colors of tumbler pigeons have been accounted for by the interaction of four genetic factors: *R*, red; *B*, black; *I*, intensity; *S*, spreading.

2. Evidence as to the nature of the factor *B* has been secured from its effect on the feather pigment with respect to (a) color, (b) manner of formation and distribution, (c) physical form, and (d) chemical properties. All of these seem to indicate a different mechanism from that which produces red pigment, rather than simply a later stage of the same process.

3. If uninfluenced by other factors, the final result of the pigmentation process in a bird carrying *B*, is the clumping of the pigment into the middle of the barbule cells. The factor *S* when present stops this clumping process and results in a 'spread' condition of the pigment. *S* may properly be considered as an inhibitive or 'stopping factor.'

4. As regards the increment of pigment substance factor *I* probably has a constant effect when acting on dilute birds of different constitutions, namely, to increase by about three times the amount of pigment produced. As regards its influence on granule shape, on the other hand, it reacts in a different manner with each combination of factors.

5. The facts concerning the granule shape in blacks suggest the possible existence of a factor not yet determined which is specifically concerned with granule shape.

6. Genetic research which is confined only to obvious characters is often superficial, and in such cases microscopic research is necessary to distinguish the independently heritable characters involved.

POSTSCRIPT

Certain features in a paper by Spöttel which has just come before the writer, deserve comment. In an analysis of "Felsentauben blau" Spöttel describes the large black spherical granules of the distal, and the smaller or even rod shaped granules of the proximal parts of the feather.

As to the relations between these two kinds of granules however Spöttel's descriptions do not coincide with those of the present writer. Spöttel speaks of a "Verschmeltzung" of several spherical granules which thus give rise to long oval, or rod-shaped granules, and pictures (M_2) a series of pigment masses which represent various stages in this fusion. A study of the developing structures furnishes unmistakable evidence that the form of the granules is determined very early in their existence, at a period in fact, long before they have attained anything like their ultimate size. The mere circumstance of what position the granules eventually come to occupy would seem unable to change their fundamental nature. It can not be denied that before cornification, while the granules are yet in a plastic condition, if they are subject to crowding, their original spherical nature will be masked—as is shown by Spöttel (fig. O_2C). It is to be noted that these granules do not fuse together in a homogeneous mass, however, and it is far less likely that linearly arranged spheres would melt together to form true rods. Pictures such as shown in figure M_2c , and d would be produced, of course, if the center granule was displaced from the linear arrangement but remained in the plane of the observer, allowing the end granules slightly to approximate one another.

Spöttel apparently has not restricted himself to feathers from birds of the Wild Rock pigeon color. He speaks (pp. 415–417) of rusty brown, reddish grey, feathers on the breast and the head regions of his birds. In these feathers, especially at the tips, he finds pigment granules varying from chocolate color to golden yellow. Granules of this color always have a spherical form. They become less numerous towards the proximal regions, being replaced by small dark or black granules, frequently rod

like in form. These are essentially the conditions which obtain in the juvenal plumage of black birds. As far as the writer can discern, Spöttel does not specify the breeding of the birds from which he obtained the material, but such conditions as he describes, and pictures in the plate have never been observed in the blues of the rock pigeon type which have been raised in our colonies.

SPÖTTEL, WALTER 1914 Ueber die Farben der Vogelfedern. II. Die Färbung der *Columba livia* nebst Beobachtungen über die mechanischen Blauverhältnisse der Vogelfeder. Zool. Jahrb. Abteilung für Ontogenie der Thiere, Bd. 38, Heft 3, pp. 357-426. 70 Text Fig. Taf. 22.

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ABBREVIATIONS

<i>a.</i> , apex of barb	<i>lt.pl.</i> , lateral plate
<i>b.</i> , barb	<i>med.cl.</i> , medullary cells
<i>b.m.</i> , basal membrane	<i>n.</i> , nuclear region of barbule cell
<i>c.bb.le.</i> , curved barbule	<i>p.</i> , pulp region (dermis)
<i>c.b.</i> , cell boundary	<i>pg.</i> , pigment mass
<i>cy.cl.</i> , cylinder cell layer	<i>pg.cl.</i> , pigment cell
<i>d.</i> , down	<i>pt.c.</i> , point at which barbule curves
<i>e.</i> , epidermal region	<i>rec.m.</i> , recurved margin
<i>h.</i> , hooks or haemules	<i>sh.</i> , feather sheath
<i>h.bb.le.</i> , hook barbule	<i>st.</i> , shaft
<i>int.cl.</i> , intermediate cells	<i>t.f.</i> , terminal fiber
<i>in.s.cl.</i> , inner sheath cells	<i>v.</i> , vane
<i>lt.sh.</i> , lateral sheet	<i>v.r.</i> , ventral ridge of barb

PLATE 1

EXPLANATION OF FIGURES

1 Cross-section of red feather showing 2 barbs with barbules; shows well how haemules interlock with clefts in curved barbules; pigmentation general in all parts; (slightly diagrammatic). $\times 100$.

2 Cross-section of a curved barbule, showing grooved nature of barbule, recurved margin and cleft; pigmented throughout with spherical granules. $\times 1100$.

3 Hook barbule from red bird (1144 C); pigmented more heavily than curved barbule from same bird. Note the narrow pigment-free areas at cell boundaries. $\times 280$.

4 Curved barbules from 1144 C; more lightly pigmented; compare with figure 3. $\times 280$.

5 Cross-section from barb from weakly pigmented red bird (93 B); ventral ridge is partly broken off. $\times 480$.

6 Portion of curved barbule from light red bird (894 B) showing thinly scattered spherical granules. $\times 1600$.

7 Portion of pigment mass from dark red feathers (5 B); granules same size and arrangement as in light red, but more closely massed. $\times 1600$.

8 Cross-section of red feather fundament before ridge formation, showing first traces of forming pigment scattered promiscuously throughout intermediate cell region. $\times 480$.

9 Diagram showing from what part of the feather (marked *x*) figure 8 was drawn.

10 Drawing from down region of a feather fundament just after ridge formation and before arrangement of lateral plates, showing generalized pigmentation (intermediate cell region). Parts of three ridges are shown. $\times 480$.

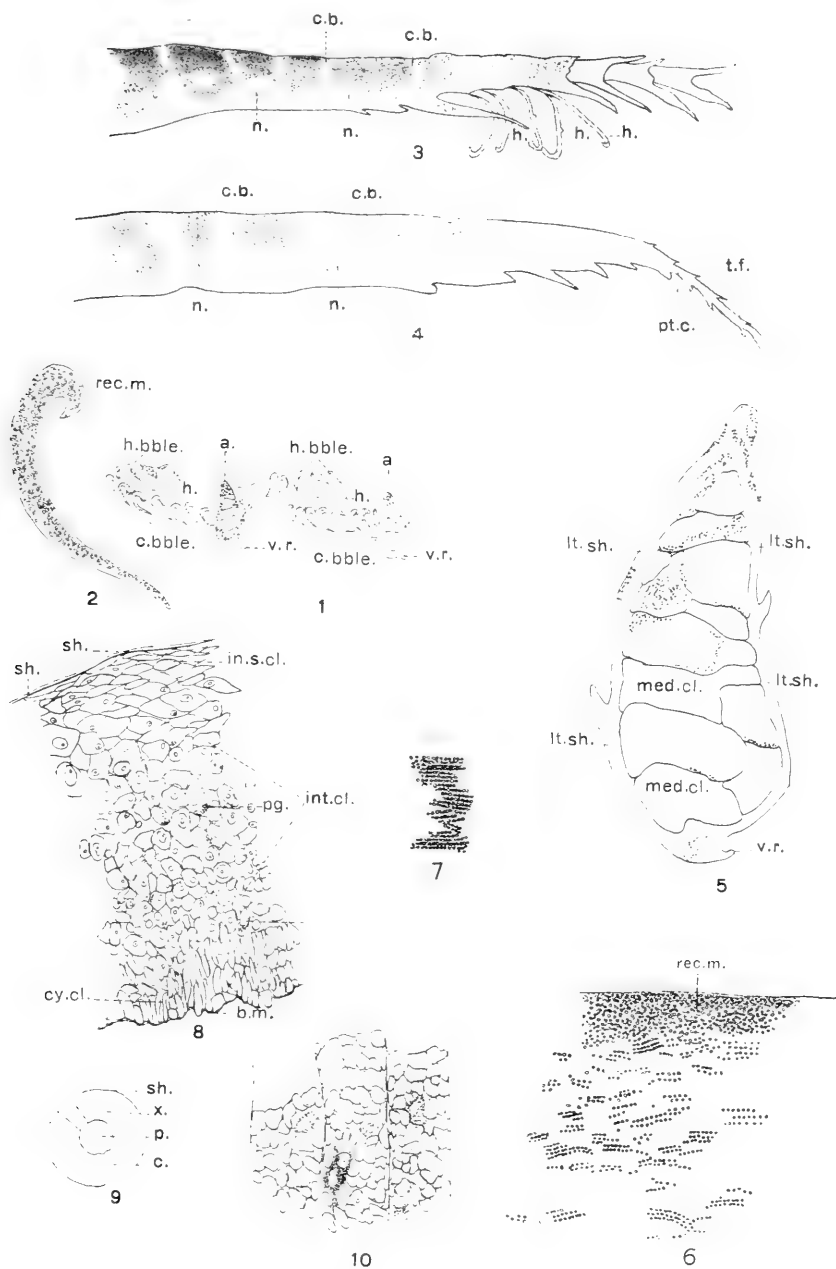


PLATE 2

EXPLANATION OF FIGURES

11 to 15 These figures are from a single feather in similar regions but at successive levels and show the several steps in feather differentiation.

11 Ridges are formed and the lateral plates well defined, two pigment cells are present. $\times 430$.

12 The cells of the lateral plates have elongated and are developing into barbules. Specialized pigment cells are not active. $\times 430$.

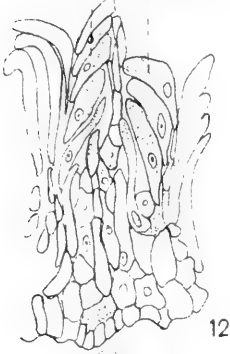
13 Barbules well developed; barb becoming defined. The pigmentation process is not concerned with pigment cells. $\times 430$.

14 Barb and barbules well defined. $\times 430$.

15 Withdrawal of barb, showing some 'residual' pigment remaining in the epidermal tissue not built into the feather. The cornified portion is here breaking away from the central supporting structure. $\times 430$.

16 Single ridge from cross-section of a feather fundament, showing copious pigmentation of feathered parts without the aid of a pigment cell. $\times 430$.

h. bble. c. bble.



h. bble. c. bble.

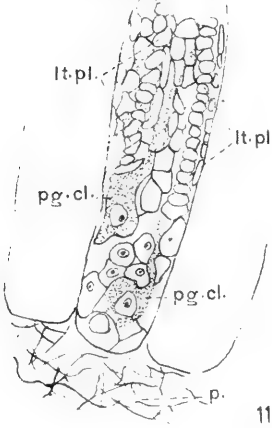


PLATE 3

EXPLANATION OF FIGURES

17 Cross-section of barb and barbules from yellow feather (76 *B*). $\times 480$

18 Portion of curved barbule from a yellow feather (19 *B*) showing the nature of feather masses, the lack of segmentation and sparse pigmentation. $\times 480$.

19 Portion of hooked barbule from a yellow feather (19 *B*), showing heavier pigmentation but also lack of segmentation. The hooks (haemules) in yellows are pigment-free. $\times 480$.

20 Feather ridge from cornified portion of fundament from yellow bird (2 *A*) showing the nature of pigment masses, also the cellular nature of the barb cortex just before complete cornification. The high attitude of the barb shown here is characteristic of flight feathers. $\times 480$.

21 Feather ridge from yellow feather fundament. A small and comparatively inactive pigment cell characteristic for yellow is shown here. Lateral plates are well formed, but the barbs are without definition. $\times 280$.

22 Cross-section of curved barbule from plum-colored feather (894 *B*) showing the inordinately large spherical granules typical of this color. $\times 1100$.

23 Portion of barbule from plum-colored feather laid flat, showing arrangement of large spherical granules. $\times 1600$.

24 Cross-section of barb or plum-colored feather (894 *B*), showing many granules within the medullary cells. $\times 280$.

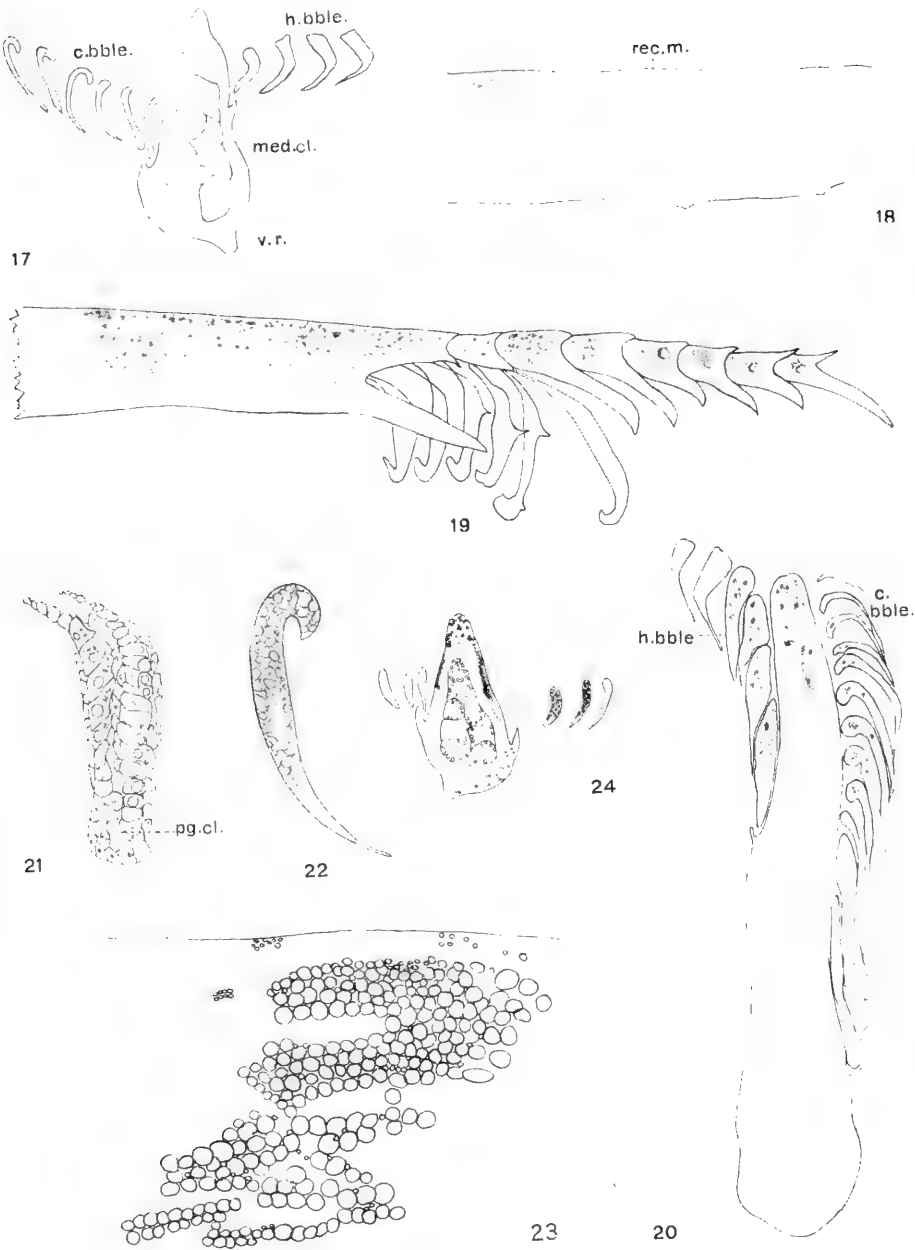


PLATE 4

EXPLANATION OF FIGURES

- 25 Portion of barbule from black bird with rod-shaped granules. $\times 430$.
- 26 Area in same barbule under higher magnification (fig. 25) showing the well defined rod granules. $\times 1600$.
- 27 Portion of barbule from black bird (1037 *B*) with the 'pebbled' effect due to spheres. $\times 430$.
- 28 Area in same barbule, highly magnified (fig. 27) showing spherical granules. $\times 1600$.
- 29 A region from feather fundament before ridge formation, showing the first appearance of black pigment. $\times 430$.
- 30 Area marked *x* in figure 29 under higher magnification, showing appearance of pigment in 'free pigmentation.' $\times 1600$.
- 31 Ridge from black feather fundament from bird 1147 *E* (poorly fixed) showing copious pigmentation and promiscuous direction of branches of pigment cells. $\times 430$.
- 32 Young pigment cell from black bird forming predominantly spheres. $\times 1600$.
- 33 Pigment cell adjoining figure 32 in same ridge but forming predominantly rods. $\times 1600$.
- 34 Portion of barbule from dun feather (959 *B*) showing sparse pigmentation and very little segmentation. $\times 430$.
- 35 Area in same barbule (fig. 34) under higher magnification showing entirely spherical granules. $\times 1600$.
- 36 Ridge from dun feather fundament, showing well developed pigment cells. Note the path of pigment branch on outside of developing row of barbules. $\times 430$.

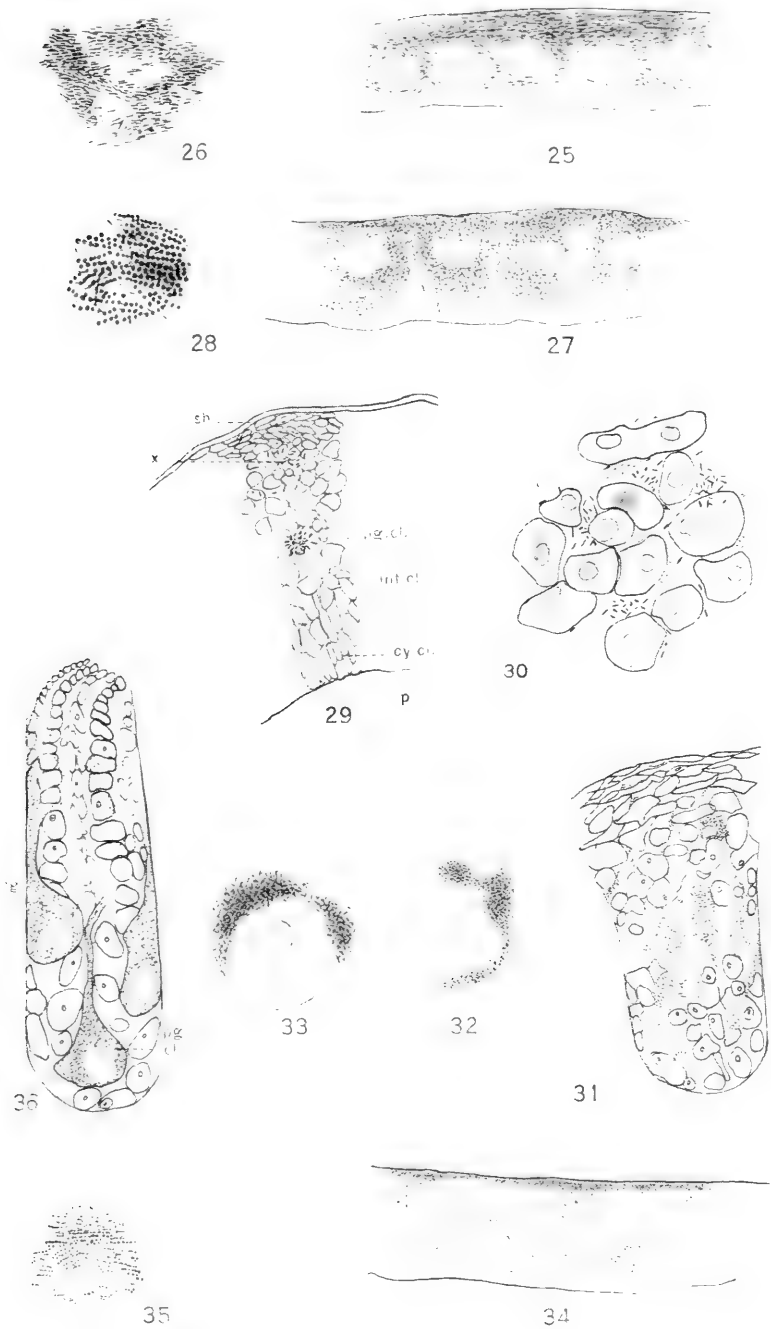


PLATE 5

EXPLANATION OF FIGURES

37 Cross-section of a blue barb and barbules (bird 1028 *A*) showing characteristic distribution of pigment. $\times 480$.

38 Cross-sections of a curved (*A*) and a hooked (*B*) barbule, showing characteristic pigment granules and manner of clumping. $\times 1100$.

39 Apex of barb from blue feather (1028 *A*). This represents all the pigment in the barb. $\times 1100$.

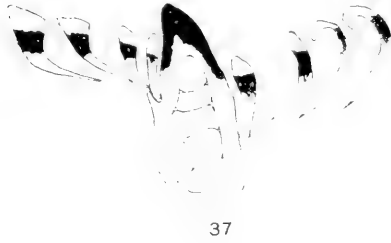
40 Portion of barbule from blue feather (265 *A*) showing the characteristic clumping of the pigment. $\times 430$.

41 and 42 Portion of pigment masses from barbule shown in figure 40, more highly magnified. $\times 1600$.

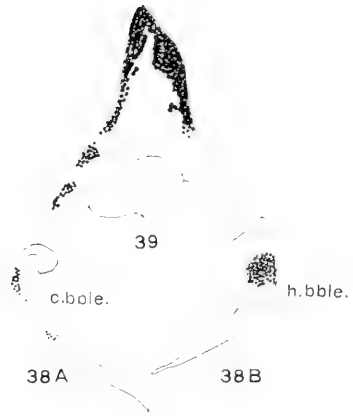
43 Portion of barbule from the base of a blue feather (1063 *B*). The base is dun in color. Rod-shaped granules are present. The pigment differs in this respect from that in dun birds. $\times 430$.

44 Cross-section of barb and barbules from dun base of blue feather (63 *B*). The portion of the feather represented here and in figure 43 appears dun only when viewed on dorsal or outer aspect, due to the spreading of the clumps in that direction only. Viewed on the inner surface they would be blue. $\times 480$.

45 Section of barb and barbules from flight feather of black bird. The portion of the barb below the barbules is pigment-free, giving to the feather viewed from this side a bluish cast. $\times 160$.



37



39

c.bole.

h.bole.

38A

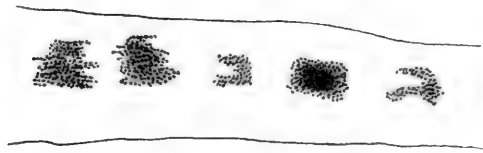
38B



42



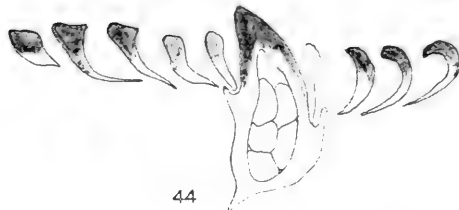
41



40



43



44



45

PLATE 6

EXPLANATION OF FIGURES

46 Lesser wing covert from blue bird. Only the distal third of the feather is blue, which fades to dun in the proximal portion.

47 Secondary covert from blue bird. Black spot is on exposed portion of feather. A row of such spots make up the black bar, a feature of the blue pattern. Shows the downy tissue found at base of such feathers.

48 Portion of ridge of feather germ from blue bird (1028 A) cut through the dun region. No spherical granules are present (fig. 44). $\times 430$.

49 Ridge from blue feather in region intermediate between blue and dun, showing lack of specificity of the tissue—all shapes and sizes of granules are present simultaneously in one ridge. $\times 800$.

50 Two contiguous pigment cells in a blue feather fundament, from region near figure 49. $\times 640$.

51 to 53 Sections from blue feather fundaments showing progressive stages in the clumping of the pigment.

51 The barbule cells have begun to elongate; the pigment is spread promiscuously throughout the cells. $\times 1100$.

52 A later stage. The pigment has withdrawn entirely from one edge of the barbule and has begun to withdraw from the outer edge. $\times 1100$.

53 Still later stage. The clumping process is well advanced and the pigment is massed in the center of the barbule cells. $\times 1100$.

54 Adjacent pigment forming areas in a blue rump feather (1113 F), intermediate cell region, before ridge formation. Regulation of granule size not absolute. $\times 1100$.

55 Showing large size granules in blue rump (bird 1113 F). $\times 1100$.

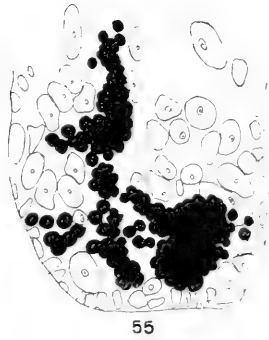
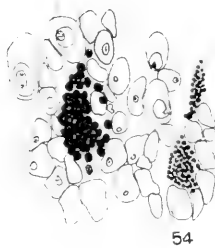
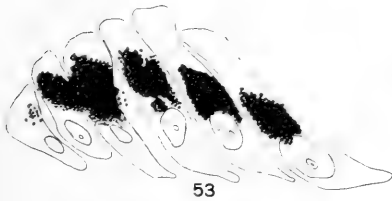
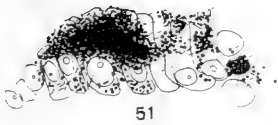
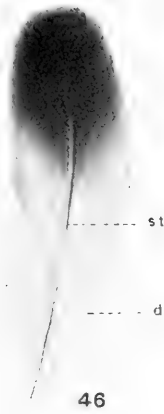
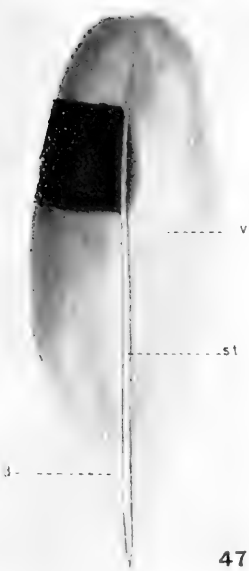


PLATE 7

EXPLANATION OF FIGURES

56 Blue rump pigment cell (1113 *F*). $\times 1000$.

57 A ridge from a silver feather fundament (1028 *B*), showing the general distribution of pigment in the silver feather. $\times 430$.

58 A hook barbule from the section shown in figure 57, enlarged. The pigment is clumped as in blue. $\times 970$.

59 A pigment cell from a silver feather fundament. The granule shape is the same as in dun. $\times 1000$.

60 Developing ridge from a silver (1175 *A*). $\times 430$.

61 Portion of barbule from silver feather (1077 *B*). Pigment masses are very faint but clumped. Barbule surface much roughened and pitted. $\times 430$.

62 A pigment mass and adjacent barbule tissue from above barbule, under higher magnification. $\times 1600$.



56



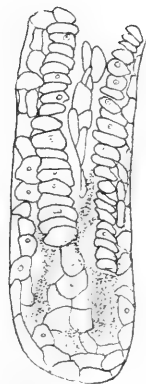
57



58



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62



61



DEVELOPMENT AND HEREDITY IN HETEROGENIC TELEOST HYBRIDS

H. H. NEWMAN

From the Marine Biological Laboratory, Woods Hole, Mass., and the Hull Zoological Laboratory, University of Chicago

ELEVEN FIGURES

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INTRODUCTION

During the months of June and July, 1914, I undertook the task of cross breeding all of the Teleost fish that could be found spawning during these months in the vicinity of Woods Hole. The excellent facilities afforded by the new laboratory building,

together with the opportunity of examining most of the fish brought in daily from the traps, made it possible for me to obtain ninety-three different crosses, seventy-eight of which are heterogenic (between different orders or families) and fifteen homogenic (between different genera of the same family or different species of the same genus). Any distinction that may be made between homogenic and heterogenic crosses must be based on an arbitrary line of demarkation. Loeb believes that there is a real difference in the character of development and heredity in the two types of hybrids which he calls homogeneous and heterogeneous, but he does not define the limits of the two types. It will be useful to consider inter-phylum, inter-class, inter-order, and inter-family crosses as heterogenic; and inter-generic, inter-specific, and inter-varietal as homogenic. Obviously there exist different grades of heterogeneity and homogeneity within the two subdivisions, and these grades will receive attention when matters of success in development and of heredity are under consideration.

In order somewhat to limit the size and scope of the present paper I have decided to deal with the seventy-eight heterogenic crosses and to leave the homogenic crosses for later communication. It was my intention to make at least two experiments with every cross but in a few cases good eggs were obtained only once and it was not possible to repeat the experiment. In most of the experiments I had the coöperation of Mr. Austin P. Larrabee, whose assistance greatly facilitated the work. The majority of the observations described in this paper have been confirmed by him although his chief interest lies in the study of the behavior of the chromatin in a number of the more favorable heterogenic crosses here dealt with. In view of the fact that Mr. Larrabee is to deal in detail with the cytological side of this subject, I shall make no statements concerning this phase of the work, but shall refer only incidentally to the cytological results of other authors.

The purpose of this paper is to present a general survey of heterogenic hybridization in Teleosts, to discuss the principal problems involved and to point out the particular parts of the

field which have seemed to me most worthy of detailed study. The one salient impression that I have carried away from my survey of this relatively extensive field is the impression of its richness. It is a new and promising line of work that offers much to the investigator who is willing to study intensively some of the more favorable crosses here dealt with only in outline.

LITERATURE ON HETEROGENIC TELEOST HYBRIDS

Comparatively few writers have dealt with fish hybrids and of these the majority have bred together rather closely related species. Of those who have described the results of inter-crossing different families or orders we may mention Appellöf, Moenkhaus, J. Loeb, Günther and Paula Hertwig, and Morris.

Appellöf ('94) is the pioneer in this field. His results and conclusions have been referred to in another recent paper (Newman '14) and only a brief statement of the crosses made by him and of their developmental success need be made. He produced but one cross between two orders: *Labrus rupestris* ♀ (family Labridae, order Haplomi) × *Gadus morrhua* ♂ (family Gadidae, order Acanthopterygii). In this cross none of the embryos went further than an early germ-ring stage with a small embryonic shield. A cross between two families of the same order was made reciprocally between *Gadus morrhua* (family Gadidae) and *Pleuronectes platessa* (family Pleuronectidae). In the cross *Pleuronectes* ♀ × *Gadus* ♂ the eggs developed normally up to early germ-ring stages but no embryonic differentiation occurred. The reciprocal cross gave entirely negative results, none of the eggs being fertilized. These two are the only heterogenic crosses that were made by Appellöf, if we use the term heterogenic as previously defined. It will be noted that in neither cross did the development proceed beyond an early gastrulation stage. Appellöf lays great emphasis on the idea that gastrulation is the most important developmental block in the embryonic life of hybrids.

Moenkhaus in 1904 described the reciprocal hybrids between *Fundulus heteroclitus* (order Haplomi) and *Menidia notata*

(order Acanthopterygii). He was not primarily interested in determining the success in development of these hybrids and therefore did not seriously attempt to rear them. His account indicates that he was unable to get the *Fundulus* egg hybrid to develop beyond the period of gastrulation and that the *Menidia* egg hybrid would not go beyond an early germ-ring stage. It was of much import to note that both the *Fundulus* and the *Menidia* types of chromosomes retained their individuality as far as late cleavage stages. In a subsequent paper published in 1911, Moenkhaus gives an account of a fairly large number of Teleost hybrids made during June and July at Woods Hole. In all twenty-eight distinct crosses were described, twenty-three of which are heterogenic. Eight of Moenkhaus' crosses are duplicates of those that I have made and fifteen of them I was unable to repeat. In general it may be said that Moenkhaus was more interested in comparing the rate of development, especially during early cleavage, in pure and in hybrid strains, than in determining their degree of success in development. No attention at all was given by him to the inheritance of paternal and maternal characters. In a few of the experiments that he was especially interested in he followed development as far as it went, but in the majority of the experiments the account closes when there has appeared a definite difference in the rate of development of the pure and hybrid strains. Comparisons between Moenkhaus' crosses and my own and detailed comments on resemblances and differences will be made in connection with each cross. It may be said in advance that Moenkhaus did not get any of his heterogenic crosses to develop very far. This failure to get any advanced embryos or larvae is rather striking in view of the quite opposite results of many of my own experiments.

A paper on "Heredity in heterogeneous hybrids" was published in 1912 by J. Loeb. In this the author deals with various Echinoderm hybrids and the following Teleost crosses: *Fundulus heteroclitus* ♀ × by *Menidia* sp. ♂; *Tautoglabrus adspersus* ♀ × *Stenotomus chrysops* ♂. It was also mentioned incidentally that a cross between *Menidia* sp. ♀ × *F. heteroclitus* ♂

had been made. The conclusion that Loeb reaches is that development in heterogenic hybrids is essentially parthenogenetic and that the spermatozoön plays only the first part of its normal rôle, that of initiating development. The formation of the embryo is stated to be purely a matter of the egg and therefore inheritance is strictly maternal. In one case Loeb cites evidence of a paternal influence in heredity but seems to have some hesitancy about presenting the data. The claim is made that the heterogenic cross in which he noted this paternal inheritance is difficult to get but I have had no difficulty in making this cross almost any day during the months of June and July. In the other three heterogenic crosses Loeb was considerably more successful in getting advanced stages than Moenkhaus had been, but he was unable to note any paternal influence except that all the embryos were more or less retarded in development and abnormal in structure, resembling pure bred embryos reared in weak NaCN in sea water. The final conclusion is that these 'parthenogenetic' embryos are pathological because of differences in the nature and rate of the chemical reactions involved. This seems to imply that the sperm does something more than initiate development, but it will be better to defer discussion of this point until the extensive data forming the body of this paper have been presented.

Quite recently Günther and Paula Hertwig have entered the field of Teleost hybridization. In addition to a number of homogenic crosses they have made a number of heterogenic crosses, all between families of the same order and none between different orders:

$$\left. \begin{array}{l} \text{Gobius jozo } \text{♀} \\ \text{Gobius capito } \text{♀} \end{array} \right\} \times \text{Crenilabrus pavo } \text{♂}$$

in both of which only pathological undifferentiated embryos were formed;

$$\text{Crenilabrus pavo } \text{♀} \times \left\{ \begin{array}{l} \text{Gobius jozo } \text{♂} \\ \text{Gobius capito } \text{♂} \end{array} \right.$$

in both of which development stopped before gastrulation; *Crenilabrus pavo* ♀ × *Box boops* ♂, in which there appeared many advanced embryos, with large well formed eyes, short tails and

considerable body pigment (these were not followed through but were preserved after two days); *Crenilabrus pavo* ♀ × *Smaris alcedo* ♂, in which most of the embryos died during gastrulation but a few developed optic vesicles and went no farther.

In connection with each of their experiments the Hertwigs studied the behavior of the chromatin in cleavage and found no chromatin elimination such as is described by Baltzer for heterogenic Echinoderm hybrids. This result agrees with Moenkhaus' earlier work on similar material, and that of Morris ('14), who studied the behavior of chromatin in *Fundulus heteroclitus* eggs fertilized with the sperm of *Tautoglabrus adspersus*. Miss Morris had no success in rearing hybrid embryos of this or the reciprocal cross.

These papers are all that I have found that deal with heterogenic Teleost crosses. Some of my own papers and those of Kammerer, List, etc., dealing with homogenic crosses need not receive attention here.

The following distinct problems seem to arise out of this survey of the literature: (1) Is success in development of hybrids correlated with the nearness of relationship of the species crossed? (2) What factors underly the differences in success in development and in heredity between reciprocal crosses? (3) Is the development of heterogenetic crosses parthenogenetic and therefore purely maternal? (4) What is the relationship existing between the degree of normal functioning of chromatin during cleavage and the development and heredity of hybrid embryos? It is hoped that the large mass of data hereinafter presented will to a large extent clear up some of these problems.

MATERIALS AND METHODS

Table 1 shows the systematic relationship of the fourteen species used. The classification of Jordan and Evermann ('00) is adopted throughout the present paper. I was unable to obtain ripe eggs of three of these species: *Poronotus triacanthus*, *Menedia beryllina cerea*, and *Morone americana*. Males

TABLE 1

Giving a list of the species of fish crossed

	GENUS, SPECIES AND COMMON NAME	SUB-ORDER	FAMILY	ORDER
1	<i>Fundulus heteroclitus</i> (common killifish)		Poeciliidae	Haplomi
2	<i>Fundulus diaphanus</i> (fresh water killifish)		Poeciliidae	Haplomi
3	<i>Fundulus majalis</i> (mayfish, striped minnow)		Poeciliidae	Haplomi
4	<i>Cyprinodon variegatus</i> (rainbow minnow, short minnow)		Poeciliidae	Haplomi
5	<i>Gasterosteus aculeatus</i> (three-spined stickle- back)		Gasterosteidae	Hemibranchii
6	<i>Apeltes quadracus</i> (four-spined stickle- back)		Gasterosteidae	Hemibranchii
7	<i>Menidia beryllina cerea</i> (silverside)	Percesoces	Atherinidae	Acanthopterygii
8	<i>Menidia menidia notata</i> (silverside)	Percesoces	Atherinidae	Acanthopterygii
9	<i>Scomber scombrus</i> (mackerel)	Rhegnopteri	Scombridae	Acanthopterygii
10	<i>Poronotus triacanthus</i> (butterfish)	Rhegnopteri	Stromateidae	Acanthopterygii
11	<i>Morone americana</i> (white perch)	Rhegnopteri	Seranidae	Acanthopterygii
12	<i>Stenotomus chrysops</i> (scup, porgie)	Rhegnopteri	Sparidae	Acanthopterygii
13	<i>Tautoglabrus adspersus</i> (cunner)	Pharyngognathi	Labridae	Acanthopterygii
14	<i>Tautoga onitis</i> (blackfish, tautog)	Pharyngognathi	Labridae	Acanthopterygii

of the first two species were abundant for a long time, but no ripe females were brought in. Only one specimen of *Morone* was secured, a ripe male that lived for some time in the aquarium and furnished excellent material for a number of crosses.

The usual methods of preventing accidental fertilization of eggs by sperm of the same species were carefully followed,

hands and instruments being washed in fresh water before each new experiment. As a farther precaution against accidental fertilization, we avoided the use of any males of a given species when experimenting with the eggs of that species. In only a few cases therefore were controls of pure bred eggs started at the same time as the hybrids. In the case of *Gasterosteus* and *Apeltes* females were abundant and males rare. In the former species males were not found until late in the season after all the crosses with the eggs of that species had been made. Hence there was no chance of accidental fertilization in this species. In the case of *Apeltes*, only an occasional small male was secured and, since no milt could be squeezed out under pressure, it was necessary to dissect out and macerate the testis in order to get any sperm. This was also true of *Cyprinodon* and only to a slightly less extent of the three species of *Fundulus*. Hence chance fertilization is out of the question in crosses involving the females of these species. In general I would add that even without any special precaution against it, accidental fertilization is highly improbable. During eight years of work with fish eggs I have never had a single embryo develop in an experiment with eggs that had not been intentionally fertilized. It should therefore be understood at the beginning that the results here reported are not to be discredited as due to accidental homogenic fertilization. We can vouch for the kind of sperm responsible for development in each cross.

The plan for presenting the data is as follows. All the crosses made upon a given species of egg are grouped together under a general heading and the various crosses are taken up in the order in which the species are listed in table 1. General remarks about the breeding habits, the peculiarities of the eggs, their rate of development and the special heritable characters of the pure-bred larvae, are made in introducing experiments with each species of egg. It seems advisable to employ in the descriptions of the different crosses the utmost abbreviation consistent with clearness of statement. It is hoped that the reader will pardon a certain amount of unavoidable abruptness in diction. When a date is mentioned it refers to one experiment

started on that day. Two or more dates mean two or more different experiments. Occasionally, two or more experiments of the same kind were made on one day and this is indicated in parenthesis after the date.

CROSSES WITH THE EGGS OF *FUNDULUS HETEROCLITUS*

This species has furnished material for a large amount of experimental and developmental work. The average diameter of the egg is about 2 mm. When first laid the eggs are covered by a sticky fibrous coat which causes them to adhere to any bodies with which they come in contact and, under laboratory conditions, to form clumps of various sizes. In order to insure normal development of either pure or hybrid embryos of this species it is necessary to dissect apart these egg clumps as soon as possible after fertilization. These eggs are remarkable for their hardness and ability to resist untoward conditions. This hardness is probably responsible to a considerable extent for the success with which this species hybridizes with many other forms. The development is comparatively slow, requiring twelve to fifteen days to reach the stage of hatching. Young larvae live well in finger bowls or small balanced aquaria. Detailed studies of the embryonic and larval chromatophores of this species and of their inheritance have been made by Bancroft ('12) and Newman ('08 and '14). Both red and black chromatophores occur on both body and yolk. The black cells are characteristically polygonal in shape and have only a few short branches. In addition they exhibit a tendency to fuse in dense groups. The red cells are intricately branched but do not fuse. In abnormal specimens both types of chromatophores may show a modification of the typical form, the blacks tending to branch and the reds to be a more solid-bodied and less branched. These characteristics of the chromatophores are strongly inherited in homogenic crosses as shown by the authors mentioned above.

F. heteroclitus ♀ × *Gasterosteus aculeatus* ♂. No males of *Gasterosteus* were found until July 4th. Two experiments

were performed July 4 and 9. The failure to secure males of the paternal species earlier in the season may be due to the fact that during the height of the breeding season of the stickleback, males stay about the nest to guard the eggs, and hence they escape the seine. In both experiments a large per cent of the eggs cleaved and developed normally up to the closure of the blastopore. Slender embryos with optic vesicles and a few somites were formed. These shortly disintegrated without developing farther. The chromatophores did not reach a definitive condition and therefore it was impossible to decide whether or not the maternal type was modified. It is my belief that this cross would have been more successful earlier in the season as both species had passed the height of their spawning activities before the experiments were made.

F. heteroclitus ♀ × *Apeltes quadracus* ♂: June 17 and 25. In the first experiment a large proportion of eggs developed normally up to an early periblast stage. Many then disintegrated but a few formed short shapeless embryos with little or no head differentiation. In none did the germ-ring go more than half way around the yolk. The second experiment was more successful. Only about 10 per cent cleaved but nearly all of these continued to develop through the germ-ring stages until the closure of the blastopore. Five embryos were developed in which the body was long and slender but no head differentiation was evident. Much pigment totally foreign to that characteristic of *F. heteroclitus* was formed on body and yolk. All chromatophores were slender and intricately branched. *No red chromatophores appeared.* This is the only case in which I have observed in *heteroclitus* egg hybrids the total suppression of the red chromatophore so characteristic of that species. I do not care, however, unduly to emphasize this observation as I was unable to repeat the cross.

F. heteroclitus ♀ × *Menedia beryllina cerea* ♂: June 19 and 29. The first experiment showed a large proportion of eggs cleaving normally. These safely passed through the germ-ring stages and many of them formed normal embryos with well developed eyes, otic vesicles, long tails, string heart; but in none was the

circulation established. Pigmentation on the body was of a modified *F. heteroclitus* type; that on the yolk seemed to be pure maternal but did not reach the definitive stage. The second experiment was similar to the first except that three individuals developed circulation in both body and yolk. These grew well for some days but gradually the heart slowed down and stopped. It may be that these embryos became infected and therefore met an unnatural fate as it is unusual for embryos that have established a circulation to fail to hatch. The embryos that reached the most advanced stage of development were strikingly like the maternal species while many of the less successful embryos showed decided traces of the paternal influence in their chromatophores. Moenkhaus described this cross using the name *Menedia gracilis*. His results are in accord with mine except that he never noted any embryos with circulation.

F. heteroclitus ♀ × *Menedia menedia notata* ♂: June 19 and 29. In the first experiment over 90 per cent of eggs cleaved and at least 50 per cent developed normally through gastrulation. The majority formed eyes with lenses, otic vesicles, a long tail, pulsating heart, etc. On the sixth day five specimens had established perfect circulation. One hatched on the fifteenth day, one on the sixteenth, and two on the seventeenth. These larvae lived for twelve weeks in a small balanced aquarium and were still alive when this account was written. I have been unable to note any differences between these hybrid larvae and those of purebred *F. heteroclitus*. The less successful embryos lived for a long time and although they did not develop a circulation continued to differentiate many organs. They show a mosaic of paternal and maternal chromatophores on both body and yolk. The heart lies in an enlarged pericardium situated in front of the head. As this pericardium grows the heart elongates and extends across from its point of attachment on the embryo to that on the opposite side of the pericardium. Though these hearts are under tension during their entire development they differentiate as though they were in normal position. The four chambers, the valves and the flexures appear in almost normal form, and the heart beats with normal rhythm although

it contains no blood. In some cases the heart becomes detached from the embryo and lies on the opposite side of the yolk; yet it still pulsates normally (fig. 1). In other cases the distal end of the heart breaks loose, leaving the free end wagging backward and forward like a dog's tail. These abnormal hearts furnish excellent examples of automatic differentiation of embryonic tissues. One is impressed with the conviction that certain tissues are predestined to form organs no matter what their

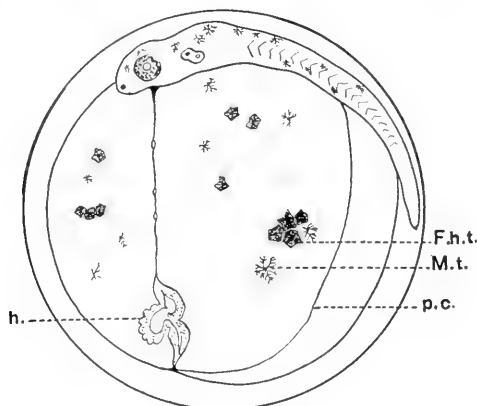


Fig. 1 A common type of hybrid embryo from *Fundulus heteroclitus* ♀ × *Menidia menidia notata* ♂ (fifteen days old). Note the heart (*h*) which, though connected with the embryo by only a delicate thread, beats with an almost normal rhythm. The heart is stretched across the abnormally large pericardium the margin of which (*p.c.*) is seen to the right. In embryos of this type both parental specific types of black chromatophore are present, the squarish *F. heteroclitus* type (*F.h.t.*) and the finely branched *Menidia* type (*M.t.*). Embryos showing this degree of paternal influence seldom develop further than this specimen.

developmental relations may be. Certainly correlative development seems unnecessary in the case of these hearts for they develop their flexures and chambers when stretched instead of being crowded as in normal development. Again when we find isolated hearts beating normally without any blood we are impressed with the extreme independence of the heart mechanism. It would be of interest to determine whether such embryonic hearts have ganglion cells. The second experiment was less successful though many well developed embryos appeared.

These displayed active muscular movements, normal heads and beating hearts; but in none was normal circulation established and in all was the paternal influence evident in the chromatophores. This cross was studied extensively by Moenkhaus who describes in detail the characters of the unsuccessful hybrid embryos. Only one specimen was seen by him to have established a circulation and even this did not hatch. This result is rather in contrast to mine in which five individuals hatched and lived for weeks. It is probable that the unusual combination of cool weather and care in segregating the best embryos accounts for my larger measure of success. Loeb ('12) evidently had little trouble in getting advanced embryos from this cross for he described cases in which a circulation had been established, but none hatched.

F. heteroclitus ♀ × *Scomber scombrus* ♂: June 18, 27, and 30. In many respects this was the most remarkable cross made on account of the striking opposition between the two species used and the marked success in development of the hybrids. All three experiments gave practically identical results, for in each some individuals developed a circulation. In the first experiment one embryo hatched and lived as an active larva for nearly three weeks. All of those that established a circulation were almost pure maternal in character, showing little or no evidence of mackerel parentage. Many of the other hybrids went on developing for at least two weeks and formed fairly normal larvae with pectoral fins that moved rhythmically. They seemed to be ready for hatching but were unable to escape from the egg membrane. A large number of the embryos that failed to establish a circulation developed a high degree of head differentiation and their chromatophores continued to grow until they reached the definitive condition. In many of these retarded embryos the chromatophores were pre-vaillingly maternal, but side by side occurred the squarish black chromatophores of *F. heteroclitus* and the intricately branched ones of the mackerel. Sometimes from a solid squarish body there ran out a slender branching offshoot. Red chromatophores appeared in nearly all embryos and, though slightly less in-

tricately branched, were evidently pure maternal. The most remarkable of all the chromatophore characters was discovered by Mr. Larrabee and confirmed by me. In a considerable number of embryos there occurred the strikingly specific green pigment cells of the mackerel. These green cells are perhaps the most highly specific of the pigment characters found in the mackerel and they never occur in pure *F. heteroclitus*, hence it would be difficult to find a clearer case of paternal inheritance in a heterogenic cross. This cross affords a graded series of individuals showing all stages of success in development from those that died during gastrulation to those that hatched and lived for weeks as normal larvae. Some of the retarded embryos show all stages of heart development seen in other crosses. Others show the pectoral fin developing out on the yolk at a distance from the embryo and in still others an extensive pericardium appeared without any heart. As *F. heteroclitus* eggs hatch in from twelve to fourteen days and those of *Scomber* hatch in less than three days we are dealing with the engrafting of two very different ontogenies and it is surprising that development is as harmonious as it is. In no case was there any hastening of the slower *F. heteroclitus* egg by the sperm of the more rapidly developing mackerel. On the contrary the development shows an early retardation and even the most successful individuals are belated in their hatching. Evidently then the rate of development is not capable of being inherited through the sperm. This cross furnishes a remarkably clear demonstration of the fact that in heterogenic crosses the maternal and paternal heredity factors are simultaneously operative. In some individuals the maternal factors seem to predominate almost to the exclusion of the paternal. In others the paternal and maternal factors seem to be about evenly balanced and in still others (very rare) the paternal factors are dominant. One specimen in particular was noted in which the yolk was covered with a delicately branched network of chromatophores of the mackerel type, none of the solid-bodied polygonal chromatophores of the maternal species being present. Not a single red chromatophore so characteristic of *F. hetero-*

clitus was present. In general it may be said that *the most successful embryos are most nearly pure maternal and that those which exhibit well defined traces of paternal influence are markedly less successful.*

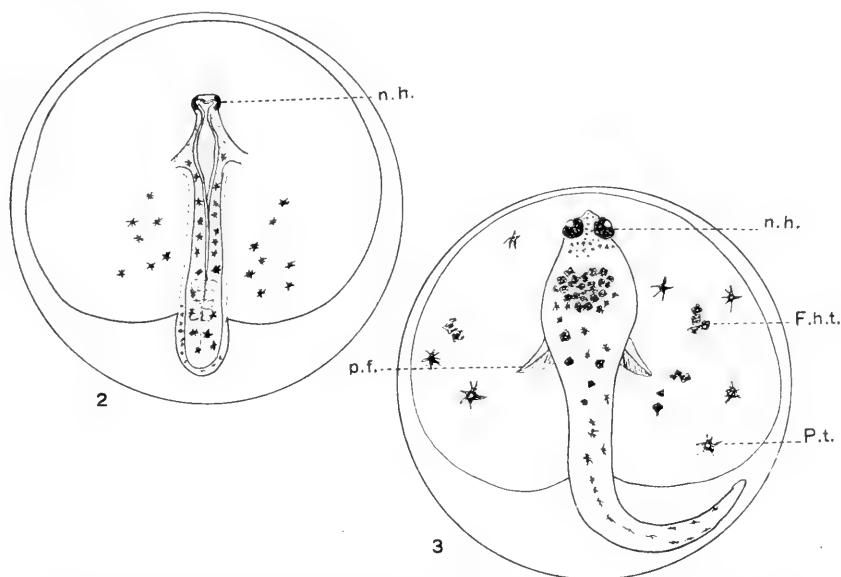


Fig. 2 Hybrid embryo from *Fundulus heteroclitus* ♀ × *Poronotus triacanthus* ♂ four days old. Note the narrow head (*n.h.*) with rudimentary eyes and the stellate chromatophores. This is the maximum stage of development reached by embryos of this cross in most of the experiments.

Fig. 3 Exceptionally advanced hybrid embryo from *Fundulus heteroclitus* ♀ × *Poronotus triacanthus* ♂. The head is still a 'narrow head' (*n.h.*) although much more advanced than usual. Note the two types of black chromatophores, the squarish *F. heteroclitus* type (*F.h.t.*) and the stellate *Poronotus* type (*P.t.*). Pectoral fins (*p.f.*) are well developed; a considerable amount of yolk has been consumed, although no circulation has been established. This embryo was fifteen days old when drawn.

F. heteroclitus ♀ × *Poronotus triacanthus* ♂: June 17, 19, 25, 26, and July 6 and 11. The results of all the experiments were strikingly similar. Nearly all the eggs cleaved normally and development though slower than the control appeared perfectly normal until the third or fourth day, when a highly characteristic type of embryo was formed, which we have called

'narrow-heads' (fig. 2). Only rarely did well defined optic vesicles appear and only in one case did eyes with lenses develop (fig. 3), and these were very small though normal in appearance. The great majority of the 'narrow-heads' showed no brain differentiation anterior to the otic vesicles. From there back however they appeared to be quite normal, forming pulsating hearts, many somites and a tail. After five or six days, however, development ceased and dedifferentiation set in, resulting in the formation of lumpy embryonic masses still retaining a spark of life. The most successful embryo appeared in the experiment of June 17 (fig. 3). It was normal in appearance until the end of the sixth day. After that time the head anterior to the otic vesicles ceased to develop and possibly diminished in size. Pectoral fins appeared and the embryo lived till July 8 when it began slowly to dedifferentiate and eventually died, without establishing a circulation. Not having been able to secure the eggs of the butter-fish I am unable to say anything definite about the paternal inheritance. It seems strange that the *F. heteroclitus* eggs should cross so much less successfully with the butter-fish than with the mackerel for both of the paternal species are equally distantly related to the maternal.

F. heteroclitus ♀ × *Morone americana* ♂: June 25. Only one ripe male of *Morone* was obtained. Early cleavage was rather irregular but development proceeded to the end of the cleavage period. No embryos formed a germ-ring, hence development ceased at the beginning of gastrulation.

F. heteroclitus ♀ × *Stenotomus chrysops* ♂: June 17, 19, 26, 30, and July 7. Scup males with abundant milt were always at hand and every opportunity for normal development was given the eggs. In four experiments no embryos reached the stage of blastopore closure and none showed any head differentiation. In one experiment, however, a considerable number of embryos succeeded in closing the blastopore and developing long slender headless bodies which were covered with small delicately branched chromatophores totally unlike those of *F. heteroclitus* but exactly like those of the scup. These embryos seemed to retain their vitality for many days after this period but did not develop farther.

F. heteroclitus ♀ × *Tautogolabrus adspersus* ♂: June 13, 19, 24, 29. There was a wide difference in the success of development observed in the various experiments. In those of June 13 and 29 80 per cent to 90 per cent cleaved and developed normally up to various germ-ring stages but none succeeded in closing the blastopore. Of these fertilized on June 24 the majority succumbed during the germ-ring stages but five closed the blastopore and produced embryos with eyes, lenses, otocysts, pulsating hearts but without circulation. On the sixth day all were dead. In the experiment of June 19 about 20 per cent of the eggs cleaved. These developed normally through the early germ-ring stages but only two succeeded in closing the blastopore. One of these failed to establish a circulation but the other continued to develop and hatched on the eighteenth day. This larva appeared to be normal and healthy but died in about a week. The other specimen that had established a circulation but failed to hatch lived considerably longer but gradually died of anemia. Moenkhaus made this cross five times but in no case did embryos develop beyond middle germ-ring stages. This result agrees with my experiments of June 13 and 29 but differs materially from those of June 19 and 24. Morris ('14) made this cross many times but did not attempt to rear the hybrids. Loeb, however, got a number of advanced hybrid embryos with "eyes, ears, fins, pigment and heart beat;" and some developed a circulation which lasted for three or four days, but none were observed to hatch.

F. heteroclitus ♀ × *Tautoga onitis* ♂: June 18 and July 3. In both experiments a large per cent of eggs proceeded normally through cleavage stages and a few reached late germ-ring conditions but failed to close the blastopore (fig. 4). Faint pigmentation appeared but it was not possible to identify the parental types of chromatophores. Moenkhaus made this cross three times with results similar to those just described.

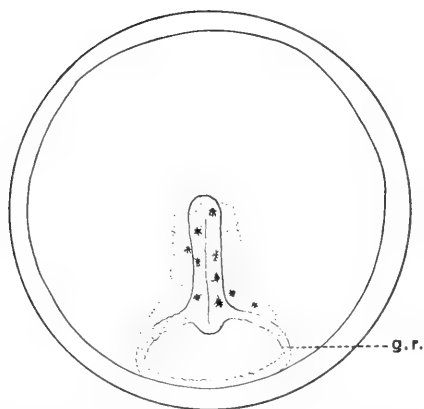


Fig. 4 Young hybrid embryo from *Fundulus heteroclitus* ♀ × *Tautoga onitis* ♂ (four days old). This represents the maximum stage of development attained in this cross. The germ-ring (*g.r.*) never completely surrounds the yolk, and very little embryonic differentiation occurs. Note, however, that chromatophores develop independently of other tissues.

CROSSES WITH THE EGGS OF *FUNDULUS DIAPHANUS*

F. diaphanus is a fresh and brackish water species closely related to *F. heteroclitus*. The egg is a little larger than that of the latter and is more transparent. The eggs are laid by the females when clasped by the males, as in *F. heteroclitus*, and adhere by means of the sticky fibrous covering to aquatic vegetation. Very few eggs of this species were available this year but in previous seasons I have crossed *F. diaphanus* with *F. heteroclitus*, *F. majalis*, and *Cyprinodon*, all of the family Poeciliidae, and with *Tautogolabrus adspersus*. In general the results are similar to those for *F. heteroclitus*, except that the egg does not seem to be so tolerant of the sperm of distantly related species. Hybrids from *F. diaphanus* eggs and the sperm of *Poronotus*, *Stenotomus*, and *Tautogolabrus*, go no farther than the germ-ring stages. The fact that *F. diaphanus* is practically a fresh water species introduces an element into the crosses that may be responsible for the lack of success in the development of heterogenic hybrids made on this species of egg. More work is needed on these crosses before any definite conclusions can be drawn.

CROSSES WITH THE EGGS OF *FUNDULUS MAJALIS*

The eggs of *F. majalis* are the largest and most heavily yolk-laden of any used in these experiments. They average 3 mm. in diameter and are of an opaque yellow color. The egg envelope is sticky but not fibrous as in other species of *Fundulus*. Presumably the eggs are laid under the stimulus of the clasping male, as I have observed upon two occasions males clasping females. I have as yet failed to find any species of Teleost which can be crossed with any marked degree of success upon *F. majalis* eggs. Even members of the same genus fail to produce any hatched larvae (Newman '08, '14).

F. majalis ♀ × *Apeltes quadracus* ♂: June 30. Only three eggs cleaved but these were isolated and well cared for. They succumbed however during late germ ring stages after forming only a broad, flat embryo without any visible differentiation.

F. majalis ♀ × *Menidia beryllina* ♂: June 30. Only ten eggs cleaved, developing short embryos with brain lobes but no optic vesicles. A short upturned tail developed although the blastopore never completely closed (fig. 5.) Chromatophores appeared but never reached a definitive stage.

F. majalis ♀ × *Menidia menidia notata* ♂: June 30 and July 1. Nearly all the eggs cleaved and developed normally up to early periblast stages, when the great majority died. Seven embryos continued to develop and were isolated and well cared for. These grew well and developed pigmented eyes, otic vesicles, many somites, pulsating string heart; but no circulation was established. The chromatophores of the two species involved are so similar that it was not possible to determine the paternal influence.

F. majalis ♀ × *Poronotus triacanthus* ♂: June 30 and July 14. In both experiments the majority of cleaving eggs developed advanced embryos with well differentiated head parts, the eyes being large and heavily pigmented (fig. 6). The blastopore never successfully closed; a short movable tail developed; the chromatophores showed no trace of the paternal influence; no heart beat was visible and no pericardium appeared. These curious embryos appeared to be specific for this cross.

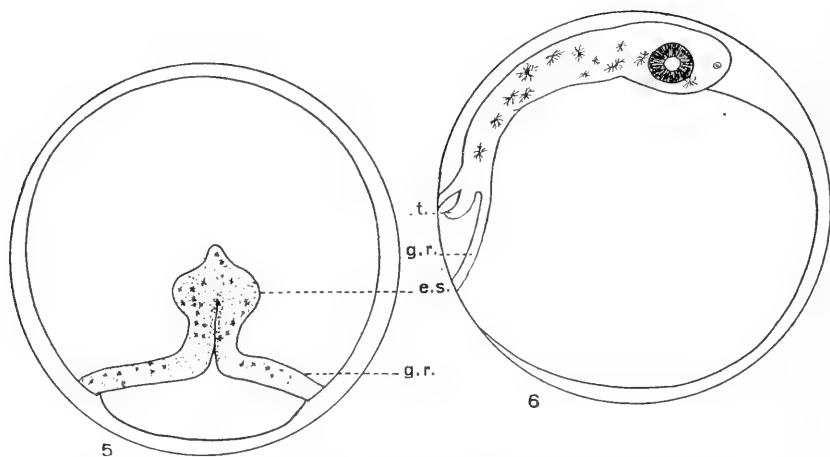


Fig. 5 Type of retarded embryo from *Fundulus majalis* ♀ × *Menidia beryllina* cerea ♂ (at maximum stage of development, five days). The embryonic shield (*e.s.*) is short and stands high above the yolk and the germ-ring (*g.r.*) is thick and well defined. Black pigment granules have begun to appear.

Fig. 6 Typical embryo from *Fundulus majalis* ♀ × *Poronotus triacanthus* ♂, showing persistent germ-ring (*g.r.*) and rudimentary tail (*t.*). This stage is a week old and at its maximum phase of development.

CROSSES WITH THE EGGS OF *CYPRINODON VARIEGATUS*

Cyprinodon is a small brackish water fish commonly called the 'short minnow' or 'rainbow minnow.' The spawning habits (described by Newman, '07) are very similar to those of *F. heteroclitus* and other members of that genus. The eggs are smaller than those of any of the *Fundulus* species, being on the average less than 1.5 mm. in diameter. They are covered with a fibrous sticky coat similar to that of *F. heteroclitus*. The yolk is almost colorless and the egg, except for its envelope, is almost as transparent as a pelagic egg. Embryos hatch in seven or eight days and the larvae live well in finger bowls. It is somewhat difficult to obtain females with ripe eggs but every season for some years past I have secured a few such individuals. In previous seasons I have crossed the eggs of *Cyprinodon* with all three species of *Fundulus* and with the cunner (Newman '14).

Cyprinodon variegatus ♀ × *Apeltes quadracus* ♂: June 29. Nearly all of the eggs cleaved normally but went no farther than early periblast stages.

Cyprinodon variegatus ♀ *Menidia menidia notata* ♂: June 29. About 40 per cent of the eggs cleaved normally. After twenty hours these had reached early germ-ring stages. Three embryos proceeded to late germ-ring stages and then broke down without closing the blastopore or developing a distinct embryonic axis.

Cyprinodon variegatus ♀ × *Scomber scombrus* ♂: July 1. Only about 10 per cent of the eggs cleaved. These developed to the end of the cleavage period but died without forming a periblast.

Cyprinodon variegatus ♀ × *Stenotomus chrysops* ♂: July 1 and 3. Nearly all eggs in both experiments cleaved and were in normal periblast stages after nineteen and twenty hours. After forty-four to forty-six hours all embryos were found to be disintegrating in early and middle germ-ring stages, without forming distinct embryonic axes.

Cyprinodon variegatus ♀ × *Tautogolabrus adspersus* ♂: June 25, 1913, and July 1, 1914. In the first experiment all embryos ceased to develop in advanced cleavage stages but in the second a large number of embryos developed normally up to late germ-ring stages showing the neural groove partly closed, primary brain vesicles, but no farther head differentiation. A little later disintegration set in and all the embryos died.

Cyprinodon variegatus ♀ × *Tautoga onitis* ♂: July 3. About half the eggs cleaved and developed normally for about eighteen hours, at which time the periblast was seen to be growing normally over the yolk but the central cells of the blastodisc showed unmistakable signs of cytolysis; none formed a distinct germ-ring.

It may be said by way of summary that *Cyprinodon* eggs appear to be ill adapted for hybridization. In all of the nine crosses the developmental block occurs early or late in the gastrulation process and no distinct embryonic differentiation occurs. The most advanced condition is reached by the inter-order cross *Cyprinodon* ♀ × *Tautogolabrus* ♂ where the first

steps in embryonic differentiation are taken. It should be especially noted that after repeated trials *Cyprinodon* refuses to develop beyond the gastrulation stages when crossed with any of the species of *Fundulus*, a genus belonging to the same family as *Cyprinodon* (Newman '14). The cause of this failure on the part of *Cyprinodon* to hybridize successfully is probably associated in some way with the constitution of the egg cytoplasm. Some specific material of the egg is doubtless so highly individual and so delicately balanced that no foreign germ plasm can successfully cooperate with it in the building up of embryonic characters. Any species of sperm seems to be able to take part in cleavage and to start gastrulation but only sperm of the same species can cooperate in embryonic differentiation.

CROSSES WITH THE EGGS OF *GASTEROSTEUS ACULEATUS*

The breeding habits of the sticklebacks are well known. The male makes a nest of grasses in which the eggs are laid and fertilized; he then guards the nest. During the best part of the spawning season no males were secured although large numbers of ripe females could be obtained at any time. Early in July, however, when the spawning season was on the wane a number of males were obtained and were easily distinguished from the females by the salmon pink coloration of the jaws, operculum, and pectoral fins. The eggs are stripped only with some difficulty. On considerable pressure they burst forth from the genital opening along with a quantity of thick mucilaginous jelly which serves to cement them into a mass or a single layer on the bottom of the vessel. After some hours the mucilaginous material becomes thinned by the sea water and may be drawn off with a pipette as a thick syrupy fluid. The eggs, however, remain rather firmly massed but may be separated by the use of some force. Many of the normally developing eggs retain the shape impressed upon them by contact with their neighbors, but this change in shape does not affect the form of the pure bred embryos.

The eggs have a rather thin envelope and measure on the average a little less than 1.5 mm. in diameter. They are of a clear yellowish tint, the yolk appearing to be of about the same density as that of *F. heteroclitus*. Pure-bred embryos hatch in about six or seven days and the larvae live well in aquaria.

Gasterosteus aculeatus ♀ × *Fundulus heteroclitus* ♂: June 15, 18 (at 10:15 a.m. and again at 4 p.m.). The three experiments gave three different results. In the first the middle cleavage stages of many showed a loose cell texture, with peripheral cells deeply separated and giving a serrated profile much like those figured by the Hertwigs, for the cross *Crenilabrus pavo* ♀ × *Smaris alcedo* ♂. (Hertwigs '14, fig. 13). In others the cleavage was normal and development proceeded in typical fashion, though more slowly than in the control, up to middle germ-ring stages. At this time the margin of the germ-ring ceases to advance and begins to retreat toward the animal pole of the egg where out of an accumulation of embryonic cells a short-bodied embryo develops that exhibits a considerable degree of differentiation. In some of these embryos well formed movable eyes occur together with large otic vesicles and brain lobes. Pigmentation was not carried very far in this experiment and the specific characters of the parent species could not be determined. In none of the embryos did a heart or pericardium develop. In the second experiment fewer embryos appeared and these disintegrated in late germ-ring stages after forming a distinct embryonic axis. The third experiment, however, was more successful than either of the others. About 6 per cent of the eggs cleaved normally and did not produce any of the crenated blastoderms described for the first experiment. More than half of the embryos developed in a normal way through the period of gastrulation and gave rise to well formed individuals with optic vesicles a little vague but with well defined otic vesicles and many clean-cut somites. Many went no farther than this but all continued to differentiate chiefly at the head end, forming darkly pigmented eyes and large swollen otic vesicles. The chief abnormality appeared at the posterior end, for the

tail was blunt and short and much too small for the body. The chromatophores appeared as a mosaic of the two parental types. One who has made a careful study of these cells in *F. heteroclitus* could not fail to recognize the foreign character of some of the chromatophores forming this mosaic. Curiously enough none of the embryos developed a heart or pericardium and hence all died of inanition after about twelve days. This cross furnishes a good example of the fact that the results of crossing two different species are not always the same. Had I stopped after two experiments I would have concluded that this cross does not go through the gastrulation period. Such considerations should make us pause in stating that any cross has certain definite limitations. I have little doubt but that some of the experiments that have gone badly with me will be successfully carried through to hatching by some other worker.

Gasterosteus aculeatus ♀ × *Fundulus diaphanus* ♂: July 14. This experiment was made too late in the season for the best results. Only about 20 per cent of the eggs cleaved. After twenty-four hours these were in early germ-ring stages which showed signs of disintegration. None lived through the gastrulation period.

Gasterosteus aculeatus ♀ × *Fundulus majalis* ♂: June 25. About 80 per cent of eggs cleaved and developed normally through the cleavage period. Many of these lived through the middle germ-ring stages, formed well defined embryonic axes, but none succeeded in closing the blastopore or in developing any distinct embryonic organs.

Gasterosteus aculeatus ♀ × *Cyprinodon variegatus* ♂: July 8 and 14. The earlier experiment was the most successful although only three eggs out of thirty-eight cleaved. These eggs developed normally through gastrulation and formed a peculiar type of elongated embryo with well formed actively moving tail but strangely twisted head parts. The anterior end of the brain was twisted under or to one side and no eyes were differentiated. Well defined otic vesicles appeared, however, and the embryo was normal from there to the posterior end. No heart or pericardium developed. The chromatophores

on both body and yolk were much like those of the maternal species. The second experiment gave a much larger per cent of cleaving eggs but no embryos developed beyond the middle germ-ring stages.

Gasterosteus aculeatus ♀ × *Menidia beryllina cerea* ♂: June 19 and 24. Both experiments gave essentially the same result except that in the first a much larger per cent (over 90 per cent) of eggs was fertilized. All developed normally through cleavage and the earlier stages of gastrulation. A few failed to complete gastrulation but most of them closed the blastopore and developed into long tailed embryos whose only defect seemed to be a certain vagueness of the head region. No distinct optic vesicles were formed but the body from the otic vesicles backward, including many clean-cut somites and well-developed tail, was normal. No pericardium or heart appeared. The chromatophores continued to differentiate and appeared to be purely maternal, although, without a knowledge of the character of the paternal elements, I am unable to pass judgment on this feature of the inheritance.

Gasterosteus aculeatus ♀ × *Menidia menidia notata* ♂: July 8 (once at 2 p.m. and again at 2:40 p.m.). The results of the two experiments were so nearly identical that only one need be described. Although a very large percentage of eggs cleaved and developed normally through the early germ-ring stages not one succeeded in completing gastrulation. In many, after the germ-ring had nearly surrounded the yolk, there was a retreat of the advancing blastoderm toward the animal pole where a piling up of embryonic cells resulted in the production of a formless embryo. The mass was always more or less clearly bilateral and somewhat larger at the anterior end. There was no indication of brain or other embryonic organs. These masses lived for a long time without undergoing any changes except in connection with their chromatophores, which continued to differentiate until they reached almost the definitive stages. No trace of paternal influence was noted.

Gasterosteus aculeatus ♀ × *Scomber scombus* ♂: June 27. About 40 per cent of eggs cleaved, the majority of them normally

but many irregularly. After seven hours, however, all seemed to be equally normal. About half of the embryos stopped developing at various stages of the gastrulation process. Fourteen specimens completed the closure of the blastopore and developed large well formed eyes, otocysts, and moving tails. Upon the fourth day five were seen to have established a more or less normal circulation, four others had actively pulsating hearts without any blood content, and five were disintegrating after reaching a somewhat less advanced stage. On the sixth day one hatched and lived a normal and active existence for ten days in a small balanced aquarium. Pure-bred embryos of this species will not live much longer than this under similar conditions. The chromatophores on both head and body of the hatched larvae were practically pure maternal in form but the less successful embryos showed a more or less pronounced influence of the paternal element.

Gasterosteus aculeatus ♀ × *Poronotus triacanthus* ♂: June 25 and 26. In the first experiment about 75 per cent of eggs cleaved and developed normally through cleavage. A few disintegrated at the end of cleavage but the majority went through the gastrulation period successfully and formed 'narrow-heads.' These peculiar embryos closely resemble similar individuals in the cross *F. heteroclitus* by *Poronotus triacanthus*. These embryos did not show any differentiation of the head region but were thoroughly normal in the body and tail regions; no pericardium or heart; the chromatophores never reached the definitive stages. The second experiment was like the first except that in it a few advanced embryos appeared with small but well developed heads and with distinct eyes and otocysts. A small pericardium and a feebly beating heart, directly beneath the body, were noted for about a day; no circulation was established in any.

Gasterosteus aculeatus ♀ × *Stenotomus chrysops* ♂: June 20, 24 and July 8. In the first two experiments the percentage of eggs developing was rather small. Those that cleaved almost invariably developed to the middle germ-ring stages but did not succeed in establishing a well defined embryonic axis. The em-

bryos turned white and the yolk opaque a few hours after development ceased. In the third experiment the results were quite different. About 60 per cent of eggs cleaved and although a large majority of the embryos died in the germ-ring stages or earlier, three succeeded in closing the blastopore and in forming embryos normal in appearance except that the head end seemed to show little differentiation. The chromatophores in all of the embryos were quite unlike those of pure *Gasterosteus* but resembled the delicately branched cells of the scup.

Gasterosteus aculeatus ♀ × *Tautogolabrus adspersus* ♂:
June 24 and 25. In both experiments a large percentage of the eggs cleaved and developed normally. About 20 per cent completed gastrulation and continued to develop up to the stage when circulation should be established, but only two embryos succeeded in developing a circulation, the others having empty pulsating hearts. The eyes in the majority of the embryos were large and heavily pigmented. Several cyclopians and two individuals with heteromorphic eyes were found; none hatched, although well cared for; even those that established a circulation died after five days. The delicately branched cunner type of chromatophore was obvious in all specimens and seemed completely to dominate the maternal type. This is the most pronounced case of the dominance of the paternal characters in heterogenic hybrids.

CROSSES WITH THE EGGS OF *APELTES QUADRACUS*

This smaller species of stickleback has presumably the same or similar breeding habits as has *Gasterosteus*. The great majority of those caught in the seine were females, only a few very small males being obtained. These are readily distinguished from the females on account of their bright red pectoral fins. The testes are minute and have to be dissected out and macerated in order to obtain the sperm. The eggs are a little smaller than those of *Gasterosteus*, averaging about 1.4 mm. in diameter. They are of a rather deep brownish amber color, the yolk being much darker and less transparent than in *Gaster-*

osteus. The process of egg stripping is rather difficult and the eggs burst out in a mass with a quantity of the same sort of gummy substance as that described for *Gasterosteus*. They may also be separated after some time, as in *Gasterosteus*. It may be said of the eggs of *Apeltes* that they hybridize much less successfully than do those of *Gasterosteus*. In no case did I obtain a hybrid from these eggs in which a circulation was established. I am inclined to attribute this lack of success to the density and indigestibility of the yolk. This topic will be discussed in detail in a subsequent section.

Apeltes quadracus ♀ × *F. heteroclitus* ♂: June 14 and July 5. In both cases only a small number of eggs cleaved and of these only three in one experiment and five in the other continued to develop beyond the cleavage period. The most advanced embryo showed the blastopore nearly closed and the outlines of small optic vesicles.

Apeltes quadracus ♀ × *Fundulus diaphanus* ♂: July 13. Nearly all eggs cleaved and developed normally to the end of the cleavage period and then died.

Apeltes quadracus ♀ × *Fundulus majalis* ♂: July 8. Nearly all of the thirty or more eggs cleaved and all but four proceeded to middle or late gastrulation stages. About twenty, after nearly closing the blastopore, formed short embryos without distinct head differentiation but with well defined though small tail. The pigmentation was not sufficiently advanced at the time of death to determine the facts about inheritance.

Apeltes quadracus ♀ × *Cyprinodon variegatus* ♂: July 7. The eggs were fertilized dry with macerated testis. A large proportion cleaved normally and seemed to be developing well after eight hours. All were dead and disintegrated the following morning after about twenty-two hours development.

Apeltes quadracus ♀ × *Menidia beryllina cerea* ♂: July 4 (once at 8:45 a.m. and again 5:20 p.m.). The results of the two experiments were quite similar except that a much larger proportion of the eggs developed in the second than in the first. The most striking characteristic of this cross is that in many cases the head end of the embryo develops independently of the tail end. The

two sometimes appear on opposite sides of the yolk with a vaguely defined sheet of embryonic tissue between (fig. 8). Sometimes the axis of the tail is nearly at right angles to that of the head. This condition appears to arise through a failure of the germ-ring to remain compressed. It seems to split apart in the mid-body region and to undergo a process of dedifferentiation. The heads end abruptly just back of the otocyst and the tail begins

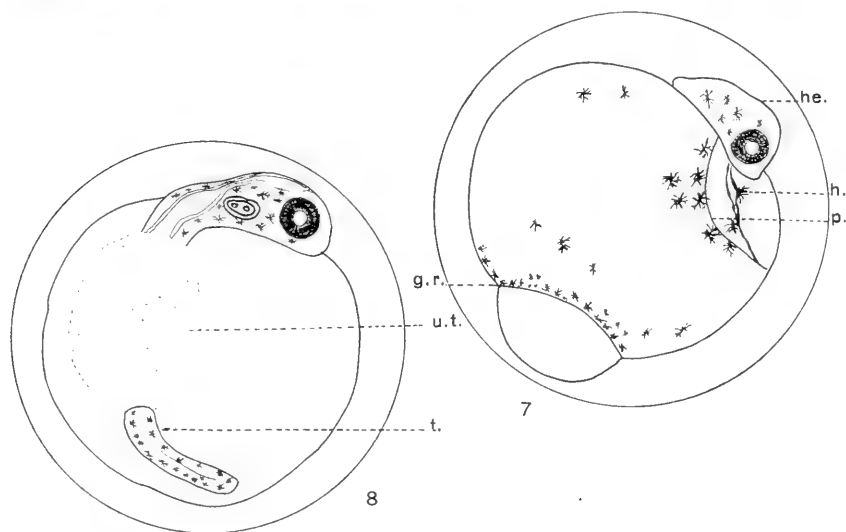


Fig. 7 One of the types of hybrid embryo from *Apeltes quadracus* ♀ × *Menidia beryllina cerea* ♂, nine days old. Note that the embryo is practically a trunkless head (*he*) with well developed eyes, heart (*h*) and pericardium (*p*). The failure to form a trunk and tail is evidently associated with the failure of the germ ring (*g.r.*) to close.

Fig. 8 Another type of hybrid embryo from the same cross as in figure 7, showing head and tail (*t*) separated by a sheet of incompletely condensed germ ring, composed of undifferentiated tissue (*u.t.*).

abruptly at some distance from the truncated head. The two parts are often on opposite sides of the yolk and at first I thought I had a peculiar type of polyembryony with one large and one small embryo developing from the single blastoderm. It is clear now however that the two bodies are merely separated parts of a single individual. The head parts are much more

highly differentiated than the tail parts, having well defined pigmented eyes and branched otic vesicles. Connected with such a head there frequently occurs a pulsating string heart. In some cases a truncated head develops without any tail on the other side of the yolk (fig. 7). Such embryos show clearly the germ-ring incompletely closed. Pigment does not develop sufficiently to show parental characters.

Apeltes quadracus ♀ × *Menidia menidia notata* ♂: July 8. About 25 per cent of the eggs cleaved and developed normally to early germ-ring stages. A very few showed the germ-ring one-half to two-thirds around the yolk and a vague embryonic shield, but none completed gastrulation stages.

Apeltes quadracus ♀ × *Scomber scombrus* ♂: June 27. About 40 per cent of the eggs cleaved but none were developing after twenty-four hours, having disintegrated during or at the end of cleavage. The male mackerel used was rather feeble at the time of the experiment and this may have been responsible for the poor success of the embryos.

Apeltes quadracus ♀ × *Poronotus triacanthus* ♂: June 30. About 25 per cent of the eggs cleaved and developed through the cleavage period. The majority proceeded to gastrulate but none were noted in which the germ-ring was more than half-way around the yolk.

Apeltes quadracus ♀ × *Stenotomus chrysops* ♂: July 7. Results practically identical with those described for the last cross.

Apeltes quadracus ♀ × *Tautogolabrus adspersus* ♂: June 24 and 26. In the first experiment nearly all eggs cleaved and developed normally to middle germ-ring stages, but none went farther. In the second experiment several embryos almost completed gastrulation but in none was the blastopore completely closed. No well-defined embryonic axis appeared in any case.

A review of the data concerning crosses with the eggs of *Apeltes* shows that this species furnishes singularly unfavorable material for hybridization, being only a little better in this respect than *Cyprinodon*. Whether the fault is with the season during which the crosses were made or in the physiological condition of the

eggs when stripped one can not certainly determine, but I believe that the factors responsible for the ill success in crossing are in no way artificial, but are correlated with some peculiarity of the yolk or other egg materials; for pure bred eggs developed quite normally when handled in the same way as the hybrids.

CROSSES WITH THE EGGS OF *MENIDIA MENIDIA* NO TATA

For hybridization experiments the eggs of this little silver-side minnow are among the most available that I have encountered. The fish, both males and females, are very abundant in the Eel Pond near the laboratory and spawn during the greater part of June and July. The eggs strip readily from the females in bunches of a hundred or more and abundant milt is easily expressed from the males. The adults live well in aquaria and may be kept on hand without showing any deterioration. in either eggs or milt for at least a week. Some of the most successful experiments here recorded have been made with those kept for five or more days. The eggs, averaging less than 1 mm in diameter, are smaller than any thus far described. In fact, they are the smallest of the shore-laid eggs dealt with in these experiments. In many respects these eggs resemble pelagic eggs, being of about the same size as the latter and resembling them in the possession of a single oil drop, which no doubt acts as a float, so that the eggs are buoyed up from the bottom although attached to weeds by means of long ropy threads. The yolk is transparent and faintly yellow in color. When the eggs are immature they strip as separate units and do not stick together. In the immature stage they are completely covered with threads of a transparent material wound in wavy bands about the chorion. When the eggs are ripe these fibers, which are seldom more than eight to twelve in number, loosen up and separate themselves from the egg surface as long twisted ropes.

All the threads are attached in a bunch to one small area of the egg which appears to be opposite to the micropyle or nearly so. When the eggs are artificially stripped these threads become intricately tangled together so that an almost inextricable

egg mass is produced. In nature, however, the eggs are apparently extruded one or a few at a time and attached to eel grass, etc., so that they float separately in the water and do not crowd one another. In order to obtain success in development with these eggs it is necessary to approximate natural conditions by dissecting apart the egg masses, a task of considerable difficulty but yielding to a little patience. If the eggs are not separated they show many unhealthy symptoms within twenty-four hours, at which time most pronounced differences in the development of different eggs are seen. Those on the surface may be in advanced gastrulation stages while those in the center show various cleavage stages. In contrast with pelagic eggs which have a rapid development, hatching in two or three days, the eggs of *Menidia*, both pure and hybrid, require nearly two weeks to hatch. The small active larvae live in the aquaria for only a few days, but hybrids usually live as well as pure bred individuals. It is a general rule among fish that the larger the egg the slower the development, and vice versa, but in *Menidia* we have an apparent exception for the egg is one of the smallest, yet the developmental period is practically as long as that of any species except *Fundulus majalis*.

Menidia menidia notata ♀ × *Fundulus heteroclitus* ♂: June 22 and 29. The first experiment was performed without dissecting apart the egg masses and, although a large per cent of the eggs cleaved and developed, none went farther than late germ-ring stages. The second experiment was much more successful. The egg mass was carefully dissected apart so that many good embryos were isolated. These were in all stages of development from early germ-ring conditions to those in which embryos had lenses, otic vesicles, somites, etc. Only the more advanced embryos continued to develop normally, although the retarded individuals gained rapidly for a while, only to succumb after a day or two. None that were distinctly retarded at the time when the egg mass was dissected apart succeeded in completely recovering from the unhealthy conditions encountered before their isolation from the mass. Many embryos succeeded in developing a normal body with a pulsating

heart, but established no circulation. These retarded hybrids show the most pronounced effects of the *F. heteroclitus* parent, not only in the presence of the squarish black chromatophores on the yolk and head, but in the possession of small but perfectly obvious red chromatophores on the yolk, a character totally foreign to the maternal species. Loeb noticed these red chromatophores in the same hybrid but expressed some hesitation in reporting their appearance, stating that they were the only paternal characters that he had noticed in heterogenic hybrids. On the fifth day six embryos established a circulation. In three the blood was abundant and in three scarce. The specimens with small blood supply died after two or more days. They showed scarcely any trace of paternal characters, no red pigment occurring on the yolk. Two embryos hatched, one in twelve and the other in fourteen days, or at about the same time as the pure bred *Menidia* embryos fertilized on the same date. These showed no paternal characters except that there occurred on the head a smaller number of black chromatophores than is normal for *Menidia*. Being less crowded they spread into a larger area, giving the impression of being larger cells. At first I was inclined to interpret these chromatophores as a modified *F. heteroclitus* character but, when later the same conditions appeared in hybrids developed from *Menidia* eggs and the sperm of *Poronotus* and of *Gasterosteus*, I came to doubt the specificity of their inheritance. Moenkhaus made this cross three times but in no case did the embryos completely close the blastopore. The most successful of his embryos formed optic vesicles but got no farther. It is probable that a failure to dissect apart the egg masses accounts for his lack of success. Loeb also made this cross and reared advanced embryos that showed the red chromatophores of the paternal species. This case he cites as an exception to the rule that heterogenic hybrids are pure maternal.

Menidia menidia notata ♀ × *Fundulus majalis* ♂: June 30 and July 8. In both experiments nearly all of the eggs cleaved. The egg masses were dissected apart after about twenty-four hours, at which time there was already a considerable variation

in the state of development of the different embryos. On the sixth day eight embryos were noted to have established a circulation, in one of which the vessels were so slender and the blood so scanty that life was maintained only for a day or two. The seven with normal blood supply lived and developed rapidly and five hatched on the fourteenth day. The two others never hatched but lived for several days longer. No trace of any paternal characters could be noted in these seven individuals but in some of the less successful embryos there was much less black pigment on body and yolk than was normal for the species. Since *Fundulus majalis* has rather delicately branched and widely separated chromatophores, this hybrid character might be interpreted as due to a paternal inheritance factor.

Menidia menidia notata ♀ × *Cyprinodon variegatus* ♂: July 1. Only about 10 per cent of eggs cleaved. After twenty-four hours these were all in early periblast stages. Although these embryos were dissected apart they were all found to be undergoing disintegration on the second day, none having completed the closure of the blastopore.

Menidia menidia notata ♀ × *Gasterosteus aculeatus* ♂: July 4 and 9. In the first experiment about 25 per cent of the eggs cleaved and were very early separated from the uncleaved eggs. The majority of the developing embryos completed gastrulation with the exception of the final closure of the blastopore. With the blastopore in the form of a slit-like opening a considerable number began to dedifferentiate but at least half of those that had entered upon the germ-ring stages successfully completed the closure of the blastopore and formed normal-looking embryos. On the seventh day twelve individuals had established a circulation, which in some was rather feeble owing to scanty blood. On the thirteenth day six hatched. The other six, those with an imperfect blood supply, showed at this time signs of disintegration. Two of these showed a few red chromatophores in the tail region, a character quite foreign to pure *Menidia* but quite typical of *Gasterosteus*. This character is not visible in any of the hatched larvae. The black chromatophores on the heads of the latter are unlike those of pure *Menidia*.

in that they are fewer, larger, and exhibit a tendency to fuse. This difference from the pure-bred embryos cannot be definitely attributed to a specific *Gasterosteus* inheritance factor, for the same state of affairs was noted in the cross *Menidia* ♀ × *F. heteroclitus* ♂. The difference, however, is obvious and may be considered as a rather general hybrid effect common to several crosses with these eggs. The hatched larvae were not as viable as the pure bred, living only about half as long under the same conditions. In the second experiment the eggs were carefully observed for only three days, at which time they seemed to be destined to have the same end result as those in the first experiment. Not having time to see the experiment through it was abandoned.

Menidia menidia notata ♀ × *Apeltes quadracus* ♂: June 21. A large proportion of the eggs cleaved normally and were dissected out of the egg mass. Only a very few safely passed the germ-ring stages, the majority stopping before the closure of the blastopore. Two however weathered the gastrulation period and grew to an advanced stage with well formed heads, pigmented eyes and pulsating hearts. As circulation was not established in either of these development ceased. The unusually dark-colored, heavy-bodied paternal type of chromatophore was obvious in both of the more advanced embryos but none of the red chromatophores characteristic of *Apeltes* were noted.

Menidia menidia notata ♀ × *Scomber scombrus* ♂: July 1. Over 80 per cent of eggs cleaved normally, but at the next observation, about twenty hours later, they were all disintegrating in late periblast stages. We were especially anxious to repeat this experiment but were unable to obtain any more mackerel.

Menidia menidia notata ♀ × *Poronotus triacanthus* ♂: June 30 and July 1. Although the first experiment showed a larger proportion of cleavages than the second, the two gave essentially the same result. The following description refers specifically to the experiment of June 30. Over 75 per cent of the eggs cleaved. The egg mass was dissected apart at an early stage. Upon the sixth day five specimens had established a

circulation. Many others had reached advanced stages but, without circulation, were unable to go on. On the fourteenth day three hatched into normal larvae which lived for several days. The other two in which circulation was noted had, at the same time, no heart beat and were dedifferentiating. Those that hatched had fewer and larger black head chromatophores than pure bred *Menidia* larvae. This is exactly the same situation as that noted in connection with the hybrids from *Menidia* eggs crossed with the sperm of *F. heteroclitus* and of *Gasterosteus*. In the second experiment only two embryos established a circulation and neither of them hatched.

Menidia menidia notata ♀ × *Stenotomus chrysops* ♂: July 1 and 3. The first experiment went better than the second, although a comparatively small percentage of eggs was fertilized. One larva developed distinct lenses in the eyes, otic vesicles and many somites, but none established a heart beat and all were dead on the fourth day. In the second experiment a much larger per cent of eggs cleaved but, although dissected apart and well cared for, they all ceased to develop in early germ-ring stages or sooner.

Menidia menidia notata ♀ × *Tautogolabrus adspersus* ♂: June 24 and 25. The two experiments were essentially identical in results. In one case about 40 per cent, and in the other case about 60 per cent, of the eggs cleaved. These developed normally at varying rates for three days and all succumbed during late germ-ring stages in which elongated embryonic axes were clearly defined.

Menidia menidia notata ♀ × *Tautoga onitis* ♂: June 3. This experiment resulted similarly to the one just described for *Tautogolabrus*. Moenkhaus made this cross and says of it that "the embryos were followed to the closure of the blastopore. The embryos were shorter than normal."

Although it has generally been supposed that *Menidia* eggs hybridize rather unsuccessfully, I have found that an increased measure of success is realized when the eggs are dissected apart from the mass in which they are stripped. I am inclined to believe that a number of the less successful experiments here

recorded could be greatly improved upon if greater care were taken of the eggs and isolation of the embryos were accomplished during the earlier cleavages. In none of my experiments did I take the precaution of isolating the eggs at so early a period. A carefully conducted series of hybrid experiment with the eggs of this species would be well worth undertaking.

CROSSES WITH THE EGGS OF *SCOMBER SCOMBRUS*

It was a disappointment to us to obtain only one good lot of mackerel eggs. This species does not stand confinement and it was necessary for Mr. Larrabee to accompany the laboratory launch to the fish traps and to perform his hybridization experiment there. He had with him the males of only two other species, *F. heteroclitus* and *Stenotomus chrysops*. Several other attempts were made to secure mackerel eggs, but, although some eggs were obtained, they were either unripe or overdue. The mackerel egg is a handsome object, characterized by a faint pinkish tinge. Like other pelagic eggs it has one large oil globule. These eggs have a diameter greater than that of any other pelagic eggs worked with, averaging about 1.2 mm. in diameter. Pure-bred embryos of this species develop well in finger bowls and hatch in less than three days as normal active larvae that live for several days in finger bowls. It was necessary to study the chromatophores of pure mackerel embryos and larvae in order to be able to judge of the paternal influence in crosses where the mackerel is the male parent. The characteristic points concerning the mackerel pigmentation are two. First the black chromatophores are exceedingly finely branched being more intricate and more delicate than those of any of the other species studied. Second there occur in certain definite places on the yolk and body large almost branchless green chromatophores, not found on any other species examined.

Scomber scombrus ♀ × *F. heteroclitus* ♂: June 30. A very large number of eggs were fertilized. Three simultaneous experiments were carried on in different vessels. The cleavage stages were perfectly normal though a little slower than in the

case of the pure-bred egg. In late cleavages it was noticed that the blastoderm had become a little irregular in most of the embryos. Shortly afterward the blastoderms began to disintegrate without beginning gastrulation.

Scomber scombrus ♀ × *Stenotomus chrysops* ♂: June 30. This experiment was begun and carried through in the same way as the one just described. The late cleavage stages were somewhat less irregular and a considerable number of embryos entered upon the period of gastrulation and went as far as the middle germ-ring stages in which a well-defined embryonic axis was noted. None however completely closed the blastopore.

It is to be hoped that more crosses with mackerel eggs will be made, for I do not consider the above experiments a fair test of their hybridizing powers. The eggs used in the experiments, however, must have been normal, for pure bred embryos fertilized at the same time from eggs taken from the same females developed normally and hatched.

CROSSES WITH THE EGGS OF STENOTOMUS CHRYSOPS

The eggs of the scup were obtained in good condition only twice but each time in great abundance. Like other pelagic eggs they go bad quickly unless stripped shortly after ripening. Females caught in nets and confined overnight or kept in live-boxes are prevented from spawning and their eggs deteriorate. Many females were secured in which the eggs though present in large quantities were all dead and disintegrating. The scup egg is among the smallest of those used in these experiments, averaging about 0.8 mm. in diameter. It is colorless and very transparent having one large oil drop. Embryonic development is rapid, the larvae hatching in less than forty-eight hours after fertilization. The larvae are incapable of living more than a day or two in finger bowls. Doubtless death is due to starvation and confinement. On hatching the larvae are very sparsely pigmented. Two kinds of chromatophores occur, one a very delicately branched black type confined to the body, and a roundish opaque yellow type occurring in definite

places about the body, one pair in front of and one pair behind the eyes; a third pair behind the otic vesicles and a number of less regularly arranged pairs on body and tail. Success in rearing pelagic egg hybrids depends on the care with which the living eggs are kept free from the decaying ones. It is my custom to skim off all the eggs found floating at the surface and to transfer them to fresh water. This, if done several times a day, gives very satisfactory results, for by this method I have been able to rear to hatching hybrids from scup eggs crossed with sperm of almost all of the species tried.

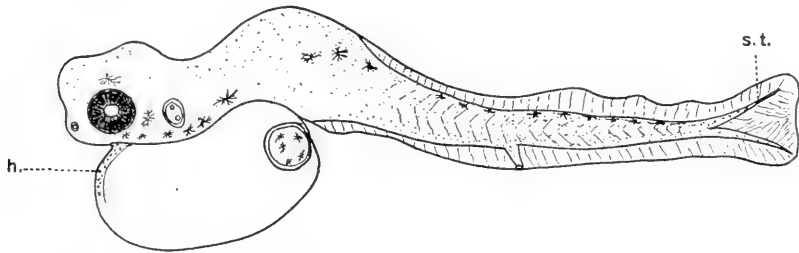


Fig. 9 Hump-backed, double-tailed hybrid larva (just hatched at three days) from *Stenotomus chrysops* ♀ × *Fundulus heteroclitus* ♂. The heart (*h*) was beating vigorously, and split tail (*s.t.*) was used vigorously in swimming.

Stenotomus chrysops ♀ × *Fundulus heteroclitus* ♂: June 26 and July 1. In the first experiment about 25 per cent of the eggs cleaved. A large proportion ceased to develop during gastrulation stages. After twenty-four hours about forty living embryos were skimmed off and were well cared for. Among the embryos that were living at this time there occurred a number of well defined abnormal types including cyclopians and individuals showing spinal bifida. After forty-six hours about twenty-five embryos were still living. A majority of these appeared to be abnormal, but in ten the heart was beating and a circulation established. At fifty-two hours two had hatched and were swimming about. These, although apparently healthy were hump-backed and one had a double tail (fig. 9). Both were found dead after three hours. This result was decidedly in contrast with the control, in which very large numbers of

perfectly normal larvae were produced. The second experiment gave results exactly like those just described, except that fewer eggs reached advanced stages. Six larvae hatched and were quite normal, living for at least twenty-four hours after hatching. I have been unable to note any *Fundulus* influence in this cross; it seems to be pure maternal in character.

Stenotomus chrysops ♀ × *Fundulus majalis* ♂: June 26. About 50 per cent of the eggs cleaved normally but the great majority of these succumbed during early gastrulation periods. The few that survived this period developed for forty-eight hours, forming advanced larvae with the head parts well differentiated but with rudimentary tails; none established circulation.

Stenotomus chrysops ♀ × *Apeltes quadracus* ♂: June 26. About 10 per cent of the eggs cleaved, nearly half of which stopped at the end of the cleavage period. The others completed gastrulation and developed into embryos with optic cups and rather vague lenses and a number of indefinite somites. After about thirty hours all embryos appeared to be disintegrating.

Stenotomus chrysops ♀ × *Menidia menidia notata* ♂: June 26. This experiment resulted almost exactly like that described above for the *F. majalis* hybrid.

Stenotomus chrysops ♀ × *Scomber scombrus* ♂: July 1. Over 80 per cent of eggs cleaved and developed normally through the cleavage period. After twenty-five hours an unusually large proportion of the embryos were alive and developing. Many of these showed the green chromatophore characteristic of the mackerel. As many as thirty hatched but only a few were normal in appearance. Others exhibited various deformities of body and tail. Conspicuous among the latter was a coiled-tail defect; all of those that hatched died in a few hours.

Stenotomus chrysops ♀ × *Poronotus triacanthus* ♂: June 6 and July 1. In the first experiment nearly 100 per cent of eggs cleaved and none showed evidences of polyspermy. After twenty-two hours only very few were alive and floating on the surface. These were in later germ-ring stages or with the blastopore just closed and the embryo developing optic vesicles. At

forty-six hours only two were still alive; these seemed to be far enough along to hatch but showed no circulation; both died without hatching. In the second experiment about 40 per cent of eggs cleaved normally and of these the majority entered upon the gastrulation period. After forty-eight hours between forty and fifty embryos had established a circulation. At sixty-five hours over twenty individuals had hatched and were for the most part alive and active. A few crooked-bodied individuals had hatched but were dead or dying when first seen. The hatched embryos seemed to be exactly like the pure scup larvae except that they hatched with a larger amount of undigested yolk. Many of these larvae were alive after two days, which is as long as pure bred larvae will live under the same conditions.

Stenotomus chrysops ♀ × *Tautogolabrus adspersus* ♂: June 26. Nearly 100 per cent of eggs fertilized. When compared with the control five hours after fertilization there was a very pronounced difference in the stage of development, there being almost twice as many cells in the pure as in the hybrid embryos. After twenty-two hours about 90 per cent of the embryos had died. After thirty hours a very few embryos were skimmed off and examined. These had apparently reached their maximum development. None of them had successfully closed the blastopore although considerable embryonic development had taken place. Well defined optic vesicles and a few somites were present, but the germ-ring, instead of closing to form the blastopore, had undergone a looping process quite different from anything observed in any other cross.

CROSSES WITH THE EGGS OF TAUTOGOLABRUS ADSPERSUS

The results of these experiments are very similar to those with scup eggs. The average size of the cunner egg is a little greater than that of the scup, being about 1 mm. in diameter. As in the scup, the eggs deteriorate quickly if the fish are kept in the laboratory. The incubation period varies with the temperature, from two to three days. The larvae do not live well in finger bowls.

Tautogolabrus adspersus ♀ × *Fundulus heteroclitus* ♂: June 14 and July 3. In both experiments large numbers of eggs cleaved and development was normal through the cleavage period and during the early stages of gastrulation. None, however, succeeded in completing gastrulation although embryos with optic vesicles were not uncommon. Moenkhaus made this cross but once and followed it only to the sixteen-cell stage. Miss Morris claims to have reared this cross for twenty-four hours, but does not mention the degree of development attained.

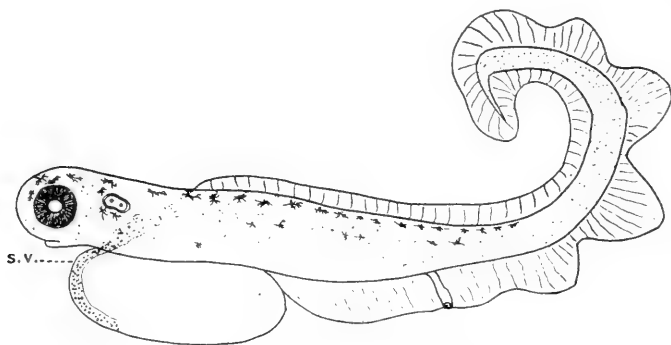


Fig. 10 Hatched hybrid larva from *Tautogolabrus adspersus* ♀ × *Fundulus majalis* ♂. Note the partially spiral tail found commonly in hybrid larvae; the sinus venosus on the yolk (s.v.) was well filled with blood and the circulation was good.

Tautogolabrus adspersus ♀ × *Fundulus majalis* ♂: June 25. About 30 per cent of eggs cleaved normally, a majority of which developed through the gastrulation stages and formed advanced embryos. Eight individuals established a circulation. On the third day two hatched both of which were abnormal in appearance with twisted tails and median fins oddly notched and wrinkled (fig. 10). They lived for only a few hours and showed no distinct evidences of paternal inheritance factors.

Tautogolabrus adspersus ♀ × *Apeltes quadracus* ♂: June 25. Only about 5 per cent of the eggs cleaved but nearly all of these developed well through the gastrulation period and twelve established a circulation. Of these eight hatched, all being more

or less abnormal. One that seemed most nearly normal differed from the pure cunner in the possession of a much shorter tail, a larger yolk sac, and a rather pronounced lack of pigment. This is the opposite of what one might expect, as *Apeltes quadracus* is the most heavily pigmented of the species used.

Tautogolabrus adspersus ♀ × *Menidia menidia notata* ♂: July 3. About 80 per cent of the eggs cleaved and nearly all began the process of gastrulation, but none succeeded in closing the blastopore. All stages in germ-ring formation were noted. Some of the embryos showed a considerable amount of head differentiation, brain vesicles and optic vesicles being distinctly defined. In all the more advanced embryos the blastopore was oval or slit-like in form.

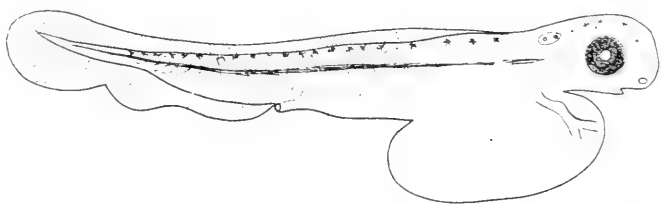


Fig. 11 The nearly normal, hatched larva from *Tautolabrus adspersus* ♀ × *Apeltes quadracus* ♂; the body is somewhat shorter than in pure bred cunner larvae.

Tautogolabrus adspersus ♀ × *Menidia beryllina cerea* ♂: July 3. About 50 per cent of the eggs cleaved, some quite irregularly due probably to polyspermy. A large number entered upon the gastrulation period. About twenty succeeded in closing the blastopore. After three days ten or more were still alive and appeared to be ready to hatch. None, however, succeeded in hatching probably on account of lack of vitality. Careful examination showed various rather pronounced abnormalities in these specimens. In a number of them the notochord was badly distorted and twisted and the brain vesicles were bent and asymmetrical. In many the somites were ill defined. Although a circulation had been established at an earlier period the heart had ceased to beat when this last observation was made.

Tautogolabrus adspersus ♀ × *Poronotus triacanthus* ♂: July 3. About 30 per cent of the eggs cleaved. Only a few individuals weathered the gastrulation period. Eight established a circulation and six hatched as normal active larvae that showed practically pure maternal characters and lived for from one to four days.

Tautogolabrus adspersus ♀ × *Morone americana* ♂: June 25. About 25 per cent of eggs cleaved normally of which a large proportion completed gastrulation and fourteen established a circulation. Four hatched, one of which lived for a day or two, the others dying in about three hours. No data on inheritance taken.

Tautogolabrus adspersus ♀ × *Stenotomus chrysops* ♂: July 4. About 80 per cent of eggs cleaved. About 200 passed the period of gastrulation and formed advanced pigmented embryos. It was expected that a very large proportion of these embryos would hatch but only about forty did so. The majority of these embryos were normal but others were twisted and deformed in various ways. Among the embryos that failed to hatch but were living on the third day were noted examples of most of the well known types of monsters, including cases of cyclopia, heterophthalmia, uniophthalmia, anophthalmia, dicephalia, spina bifida, microcauda and many other deformities. A great many specimens were found that had partially hatched, a tail being thrust out of the egg membrane but the body being still enclosed. Any one interested in the study of monsters would find this cross excellent material for investigation.

CROSSES WITH THE EGGS OF TAUTOGA ONITIS

The Tautog egg is, so far as size and appearance go, identical with that of the cunner but in ability to hybridize it differs very materially from the latter. Although the eggs used were evidently in good condition, as evidenced by the fact that the pure bred larvae hatched in large numbers, none of the crosses succeeded in completing the process of gastrulation. All the crosses were made from the eggs of one large female and gave

practically identical results in each case. The following species were used as sperm parents: *Tautoga onitis*, *Fundulus heteroclitus*, *Menidia menidia notata*, *Poronotus triacanthus*, and *Tautoglabrus adspersus*. Whether or not the eggs of the *Tautog* are always as unsuccessful in crossing remains to be seen. We were unable to obtain good eggs of this species a second time. Moenkhaus describes a cross between *Tautoga onitis* and *Menidia menidia notata* in which all embryos died before or during germ-ring stages. In some the germ-ring had spread about two-thirds over the yolk.

DISCUSSION OF DATA

Success in development and systematic relationship

A survey of the results of these crosses shows that a large percentage of even the most heterogenic hybrid strains produce embryos with circulation and that a somewhat smaller percentage give rise to active free-swimming larvae. A list of those crosses in which a circulation was established or larvae hatched will be useful for reference (table 2). Of these crosses thirteen are between two different orders, four between different sub-orders of Acanthopterygii, and one between two families of the sub-order Rhenopteri. This is the first time that heterogenic Teleost hybrids have been reared to hatching. I am unable to offer an explanation of the failure of other workers to get similar results, but I am convinced that even better results can be obtained by any one who will take reasonably good care of the embryos. There appears to be no reason to doubt that certain of the hybrid larvae could be reared in aquaria and, if properly cared for and fed in orthodox fashion, carried on to maturity. The data here given seem to furnish a distinct argument against the idea that success in development of hybrids is correlated with the nearness of relationship of the species used. Particularly does this idea fail when it is recalled that a considerable number of homogenic crosses exhibit much less success in developing advanced embryos than many of these most heterogenic strains. Further light may be thrown upon this point

TABLE 2

SPECIES CROSSED	CIRCULATION ESTABLISHED IN	NUMBER HATCHED
Fundulus heteroclitus ♀	3	0
Menedia beryllina cerea ♂		
Fundulus heteroclitus ♀	5	5
Menidia menidia notata ♂		lived 3 weeks
Fundulus heteroclitus ♀	5	1
Scomber scombrus ♂		lived 3 weeks
Fundulus heteroclitus ♀	1	1
Tautogolabrus adspersus ♂		
Gasterosteus aculeatus ♀	5	1
Scomber scombrus ♂		lived 10 days
Gasterosteus aculeatus ♀	2	
Tautogolabrus adspersus ♂		
Menidia menidia notata ♀	8	5
Fundulus majalis ♂		
Menidia menidia notata ♀	12	6
Gasterosteus aculeatus ♂		
Menidia menidia notata ♀	5	3
Poronotus triacanthus ♂		lived several days
Stenotomus chrysops ♀	10	2
Fundulus heteroclitus ♂		died in a few hours
Stenotomus chrysops ♀	50	25
Scomber scombrus ♂		lived from 2-12 hours
Stenotomus chrysops ♀	40	20
Poronotus triacanthus ♂		lived about 2 days
Tautogolabrus adspersus ♀	8	2
Fundulus majalis ♂		lived only few hours
Tautogolabrus adspersus ♀	12	8
Apeltes quadracus ♂		lived 2 days
Tautogolabrus adspersus ♀	8	6
Poronotus triacanthus ♂		lived 1-2 days
Tautogolabrus adspersus ♀	14	4
Morone americana ♂		lived 2-4 hours
Tautogolabrus adspersus ♀	100	about 40
Stenotomus chrysops ♂		lived 2-24 hours

by an examination of a long list of unsuccessful heterogenic crosses, those that did not survive beyond the germ-ring stages. In table 3 appears the list of these crosses, given in the order in which they appear in the descriptive part of the paper. One should, however, especially note the fact that in all these crosses, except that between *Fundulus heteroclitus* ♀ × *Tautoga onitis*

TABLE 3

EGG PARENT	SPERM PARENT
Fundulus heteroclitus.....	{ Morone americana Tautoga onitis
Fundulus diaphanus.....	{ Poronotus triacanthus Stenotomus chrysops Tautogolabrus adspersus
Fundulus majalis.....	Apeltes quadracus { Apeltes quadracus Menidia menidia notata
Cyprinodon variegatus.....	{ Scomber scombrus Stenotomus chrysops Tautoga onitis
Gasterosteus aculeatus.....	Fundulus diaphanus { Fundulus diaphanus Cyprinodon variegatus
Apeltes quadracus.....	{ Menidia menidia notata Poronotus triacanthus Stenotomus chrysops
Menidia menidia notata	{ Cyprinodon variegatus Scomber scombrus
Scomber scombrus.....	{ Fundulus heteroclitus Stenotomus chrysops
Tautogolabrus adspersus	Menidia menidia notata { Fundulus heteroclitus Menidia menidia notata
Tautoga onitis.....	{ Menidia beryllina cerea Poronotus triacanthus

♂, only one experiment was carried out. It is highly probably that had several experiments been made with each cross some of those listed here would have to be transferred to table 2, but, making due allowance for the likelihood that a considerable number of these crosses would result in more advanced stages if repeated a number of times, it is likely that a very large residue of unsuccessful crosses would remain. Of these least successful crosses here listed twenty are between different orders, four between different sub-orders of Acanthopterygii, and two between different families of the same sub-order. These proportions do not differ materially from those that held for the most successful crosses. In other words, it would appear that

among heterogenic crosses success in development is not correlated with the nearness in relationship of the two species used. Even when we consider homogenic crosses it is found that certain hybrid types, as for example those between *Cyprinodon* and the various *Fundulus* species (Newman '14), are decidedly unsuccessful. These two genera, nevertheless, are closely allied members of the same family, the Poeciliidae. I was never able to get any of these crosses to develop beyond gastrulation. Furthermore, it is not even always true that crosses between two species of the same genus develop as successfully as do some of the most heterogenic crosses. It is well known, for example, that when the eggs of *Fundulus majalis* are fertilized by the sperm of *F. heteroclitus* or *F. diaphanus*, no embryos hatch and many of them fall far short of this stage. Contrast this situation with the fact that so many crosses between distinct orders give a fair number of viable larvae and large numbers of advanced embryos and it will become evident that we must look to some other factors than those involved in systematic relationships for an explanation of the varying degree of success in the development of hybrid embryos.

Factors governing success in development of teleost hybrids

Among the most striking general facts that arise out of the data given above are: that the eggs of some species never hybridize well, while those of others appear to have a high capacity for crossing with almost any other species. The eggs of *F. majalis*, for example, develop poorly even when fertilized with the sperm of a closely allied species of the same genus, for I have never seen a hybrid larva hatch from the egg of this species. A glance at the other crosses with *F. majalis* eggs shows that none of the heterogenic hybrids even develop a circulation. In fact most of these hybrids ceased to develop in the germ-ring stages. In other papers (Newman '08, '14) the ill success of *majalis* egg hybrids was attributed to the large size of the egg and the indigestibility of the yolk. There is apparently something about the yolk of this egg that renders it difficult material for hybrid

embryos to break down. It evidently digests slowly even in pure-bred embryos for it requires three weeks or more for them to hatch. No success was obtained with heterogenic hybrids from the eggs of *F. diaphanus*. Since, however, I had so few eggs and made only one experiment on each of the few crosses tried, I do not care to lay much stress upon the results here recorded. I am convinced, however, that even under the best conditions it will be found that this species hybridizes much less successfully than its nearest relative *F. heteroclitus*. *Cyprinodon* seems to furnish another example of a species the eggs of which cross badly with even its closest relatives. I have never observed an advanced hybrid embryo derived from a *Cyprinodon* egg although these eggs fertilize and cleave normally for a considerable time. The eggs of *Apeltes* give results that remind one strongly of those obtained with the eggs of *F. majalis*, for they cross poorly even with the most closely related species, *Gasterosteus*, and in heterogenic crosses they never go beyond an early embryonic stage. These eggs are of the same size and are laid in the same way as those of *Gasterosteus* yet there is a very marked difference in the developmental success of these two species of *Gasterosteidae*. The only notable difference between the eggs of these two species is in the color of the yolk, that of *Apeltes* being brownish amber and that of *Gasterosteus* being a much lighter yellow. The color difference is doubtless associated with density as well as with chemical composition. The darker, more opaque, yolk of *Apeltes* is evidently harder to digest than the lighter more transparent yolk of *Gasterosteus* and is therefore less favorable material for hybrid development.

Although some pelagic eggs such as those of the scup and cunner hybridize very successfully, others, as those of the Tautog and the mackerel have poor success. I would not be willing, however, to go on record as predicting that the degree of success described represents the maximum of success obtainable with these crosses, for only one lot of good eggs of each of the last two species was obtained. It cannot then be said that mere egg size is a controlling factor in determining the success of development in hybrids, although in general it seems to be true

that small eggs with little yolk hybridize better than large eggs with voluminous yolk. The three species that hybridize with the largest degree of success have small eggs: those of *Tautoglabrus*, *Stenotomus* and *Menidia*. Of these the first two are pelagic and the last is a type laid on or near the bottom. The eggs of *Menidia* would, I am convinced, hybridize very much more successfully if they could be scattered when they are stripped instead of being bunched into dense masses. This tendency to form egg aggregates is a mere mechanical factor which has nothing to do with the hybridizing capacity of the species, yet may very materially affect the results. I am inclined to believe that, making due allowance for this mechanical hindrance, the eggs of *Menidia* cross more successfully than those of any others species here dealt with. Considering the rather large size of the eggs of *Fundulus heteroclitus* and the fact that they, like those of *Menidia*, have a tendency to adhere in bunches, these eggs hybridize with rather marked success. For crosses produced respectively with the sperm of *Menidia beryllina cerea*, *Menidia menidia notata*, *Scomber scombrus*, and *Tautoglabrus adspersus*, give embryos in which the circulation is established and, with the exception of the first cross, produce larvae. Only one out of eleven heterogenic crosses with the eggs of this species failed to develop fairly advanced embryos. *F. heteroclitus* eggs are well known to be extraordinarily hardy, thriving even under the most adverse conditions. Normal larvae have been known to exist even in the foulest sea-water and to thrive in fresh as well as in brackish water. It is probably in consequence of the hardness of these eggs that hybrid larvae derived from them develop so well and reach such advanced stages; for a robust egg could conceivably overcome the difficulties involved in inharmonious germ plasms. In view of these facts the conclusion seems to be justified that *the chief factors making for success or failure in hybrid development are associated with certain specific characters in the egg, of which amount, composition and density of yolk, hardness or delicacy, and certain mechanical advantages or disadvantages, play a leading rôle.*

There are certain indications also that some species of sperm are better adapted to hybridization than others. The sperm of *F. heteroclitus*, for example, as well as that of both species of *Menidia* and of *Poronotus* give a much better set of hybrids than does that of *Stenotomus*, of *Cyprinodon* or of *Tautoga*. This difference is not correlated with the amount of milt or with the freedom with which it may be stripped. There must be then some specific peculiarity that renders certain sperm good for hybridization and others bad. Possibly the size of the sperm head may be a factor but there are no data on this point. One of the respects in which Teleosts differ most widely is in the rate of development. Some species develop to hatching in two days and others require three weeks or more. One would be inclined to expect *a priori* that when two species with the same developmental rate are crossed we would obtain a more harmonious end result than when two species with a very different rate are used. This is true, however, only to a very limited extent. The various species of pelagic eggs which hatch in two or three days unquestionably hybridize together with more general success than do these species when crossed with forms that have a slow developmental rhythm. But there are cases which seem to form an exception to this rule. *Menidia* eggs, for example, although requiring two weeks for development, hybridize well with *Poronotus*, which hatches in about two days; *Fundulus heteroclitus* hatching in two weeks crosses successfully with *Scomber scombrus* which hatches in two to three days. Again, the eggs of *Scomber scombrus* (hatching in two to three days) do not develop beyond the germ-ring stages when crossed with *Stenotomus* which has the same developmental rhythm. On the other hand, *Fundulus heteroclitus* and *Menidia menidia notata*, both of which have about the same developmental period (approximately two weeks), produce viable larvae from both reciprocal crosses although they represent two distinct orders.

We may conclude then that *success in hybrid development is not correlated with nearness of relationship but with certain specific*

characters of the germ cells, especially of the eggs, of which yolk composition is probably the most important and rate of development of secondary significance.

Factors governing the differences in success of reciprocal crosses

It has been noted by many writers that reciprocal crosses differ not only in success of development but also in rate of development and in inheritance. The extensive array of reciprocal crosses here described furnishes material of importance for the solution of the problems involved in this situation. In table 4 are listed all the heterogenic crosses that are reciprocal. A

TABLE 4

EGG PARENT	SPERM PARENT
	Menidia menidia notata
	Gasterosteus aculeatus
	Apeltes quadracus
Fundulus heteroclitus.....	Stenotomus chrysops
	Scomber scombrus
	Tautogolabrus adspersus
	Tautoga onitis
Fundulus diaphanus.....	Menidia menidia notata
	Apeltes quadracus
Fundulus majalis.....	Menidia menidia notata
	Stenotomus chrysops
Cyprinodon variegatus.....	Menidia menidia notata
	Gasterosteus aculeatus
	Apeltes quadracus
Gasterosteus aculeatus.....	Menidia menidia notata
	Tautogolabrus adspersus
Apeltes quadracus.....	Menidia menidia notata
	Stenotomus chrysops
Menidia menidia notata.....	Stenotomus chrysops

review of these crosses shows that there is not always a marked difference in success of reciprocals. For example, *Fundulus heteroclitus* and *Menidia menidia notata* cross equally well both ways. The same is true for the last cross listed, *Stenotomus* by *Tautogolabrus*. Certain other reciprocals go badly both ways, as for example *F. heteroclitus* by *Tautoga* and *Cyprino-*

don by Apeltes. Certain others go moderately well both ways as *F. heteroclitus* by *Gasterosteus* and *F. heteroclitus* by Apeltes. The majority of these crosses show a marked difference in the developmental success of reciprocals. All the crosses involved with *Fundulus majalis* were less successful when the egg of this species was used than when *F. majalis* was the male parent. The same was true also for *Cyprinodon* which gives exceedingly poor results when its eggs are used but makes a number of successful combinations when its sperm is employed. The reverse is in general true for *Stenotomus* which gives better results when used as the egg parent than as the sperm parent.

Thus we are led to the inevitable conclusion that this difference between reciprocals is dependent upon the general hybridizing capacities, irrespective of systematic relationship, of the two types of parental germ cells used. When for example two species are crossed, the egg of one of which had a high and the other a low capacity for hybridizing, we expect the hybrid from the better egg to develop more successfully than that from the poorer egg. A concrete case is furnished by the cross between *Fundulus majalis* with a large egg that has a low hybridizing capacity, and *Menidia* a species with a small egg of marked hybridizing capacity. The result is that the *F. majalis* egg hybrid ceases to develop in germ-ring stages, while the *Menidia* egg hybrid produces a number of viable larvae. *As was found to be true for crosses in general, the underlying cause for differences in developmental success of reciprocal crosses has its seat in the nature and amount of the yolk and has nothing to do with the relationship.*

Inheritance in heterogenic hybrids

In a recent paper Loeb ('12), after a study of a few Teleost crosses derived chiefly from the egg of *Fundulus heteroclitus*, comes to the conclusion: "that the formation of the embryo is purely a matter of the egg and that the main function of the spermatozoön is the causation of the development of the egg. If we may express this statement in the form of a paradox we

may say that fertilization is primarily and essentially artificial parthenogenesis." This radical conclusion was reached as the result of observations on the inheritance of chromatophores in heterogenic hybrids. In all the hybrids made with the eggs of *Fundulus heteroclitus* Loeb sees only the maternal types of chromatophore. This species has two types of these pigment cells, black and red, and these occur on both body and yolk. Now black chromatophores are common to all Teleost embryos, and red cells occur in a number of the species that I have dealt with. The specific differences lie in peculiarities of branching, in tendencies to fuse or to remain separate and in other minor characters. One has to become very familiar with the specific peculiarities of these chromatophores in order to be able to judge of their inheritance. Each type has its own developmental history and each is much more generalized (less specific) in form during the early stages than after it has reached the definitive condition. The definitive black chromatophores of *F. heteroclitus* are large polygonal bodies unlike those of any other species observed. They exhibit only a slight tendency to branch and a strong tendency to fuse in groups. Chromatophores with long slender branches are totally foreign to the healthy *F. heteroclitus* embryo. Branching, however, may be induced experimentally by adding a small amount of NaCN to the sea water as was shown by Loeb. Although it must be admitted that chromatophores can be made to branch under the influence of certain chemicals I have never noted this condition in even the unhealthy pure-bred larvae reared under laboratory conditions. Consequently, when in certain hybrid embryos a well defined type of branching chromatophore appears either to the exclusion of the polygonal type or side by side with the latter, its occurrence cannot be adequately explained as the result of ill health in the embryo due to deleterious effect of foreign germ plasm. In many ways the chromatophores would seem to furnish unsatisfactory material for the study of paternal and maternal inheritance, because these structures are so variable in habit. One must therefore rely for a demonstration of paternal influence upon certain cases that are beyond the range

of controversy. The following are some of the heterogenic crosses in which there is positive proof of the foreign spermatozoön playing a rôle in heredity. The case of *Menidia menidia* notata ♀ × *Fundulus heteroclitus* ♂ was cited by Loeb as follows:

While it is the rule that in the case of heterogeneous hybridization heredity is purely maternal it is possibly not without exception. I have, however, thus far found only one paternal characteristic that is possibly transmitted to a heterogeneous hybrid. The yolk sac of *Fundulus heteroclitus* forms branched red chromatophores which are not found on the yolk of *Menidia*. In two eggs fertilized by the sperm of *Fundulus heteroclitus* a few red chromatophores were observed. It is difficult to get this cross and I give this observation with some hesitancy.

I have obtained this cross several times and could have made it as often as I wished, for the two species were spawning through most of June and July and hybridized well together. I am able to confirm Loeb's observation of red chromatophores on the yolk of the *Menidia* egg hybrid. They occur in many embryos that reach a moderately advanced condition but seem to be lacking in the most successful embryos, which are nearly pure maternal. Perhaps the best cross to show paternal heredity is that resulting from *F. heteroclitus* ♀ × *Scomber scombrus* ♂. These two species have chromatophores of as distinct and opposite types as possible. The blacks and reds of *F. heteroclitus* have been described. The mackerel has a black type of extreme delicacy with scarcely any body to the cell and with many slender branches, and a highly characteristic and quite unique green type. Now in many of the moderately successful hybrid embryos, chromatophores, of the squarish sort characteristic of *F. heteroclitus*, lie side by side with those of the finely branching type characteristic of the mackerel. In other hybrids the body of certain chromatophores may be of the pure *heteroclitus* type but this body may give off one or more long branches of the delicate sort characteristic of the mackerel. Loeb believes that the modified chromatophores of heterogenic *Fundulus* egg hybrids are due to lack of oxygen, but it is hardly likely that the oxygen conditions are so different for two adjacent cells as

to make one assume the finely branching form and leave the other unchanged.

We must conclude then that the condition here presented is to be interpreted as a mosaic of paternal and maternal inheritance units. No more remarkable example of paternal influence in heterogenic crosses could be asked for than that seen in connection with the green and the red chromatophores of the two species in question. Remembering that the green type never occurs in *F. heteroclitus* and that the red type always occurs in this species, it is startling to find in a considerable number of the hybrids from *F. heteroclitus* ♀ and *Scomber scombrus* ♂ unmistakable green chromatophores, and in others a total lack of red yolk chromatophores. The embryos that lack red cells have, in addition, no blacks of the *F. heteroclitus* type, but show a fine network of branching chromatophores all over the yolk. Here we have then the transmission of a positive paternal character on the one hand, and the suppression of a positive maternal character on the other, neither of which can be explained on any other basis than that the foreign spermatozoön exercises an hereditary influence and that, therefore, Loeb is wrong in supposing that this kind of development is purely maternal and essentially parthenogenetic. Many other evidences of paternal heredity are cited in the description of different crosses and need not be recapitulated here. It is commoner to find paternal characters in heterogenic crosses than to note its absence.

The findings of Moenkhaus, of the Hertwigs, and of Miss Morris, that there is no elimination of chromatin in heterogenic Teleost hybrids, are in line with the revelations of this paper concerning the influence of the paternal element in heredity. In most cases the maternal influence is more pronounced than the paternal but perhaps we should expect this because of the larger deutoplasmic contribution of the egg. But there is nearly always a series of embryos ranging from those showing a very marked paternal dominance to those showing little or no trace of it. As a general rule I have found that *when the paternal influence is strong the embryo fails to reach its maximum development and that all or nearly all of the most successful hybrids are*

predominantly maternal. In a number of cases hybrid larvae differ in their inheritance characters from the pure bred larvae but the differences are of a generalized character not definitely referable to the paternal species. This is true of the hybrids from *Menidia menidia notata* eggs and the sperm of *F. heteroclitus*, *Poronotus* and *Gasterosteus*. In all three of these hybrid strains the hatched larvae show a few large solid chromatophores on the head instead of the numerous branching bodies characteristic of the *Menidia* larvae. This difference between pure and hybrid forms is very striking but I do not understand its significance.

It seems to be true, within limits, that only the most successful hybrids are preponderant maternal and that the less successful larvae in nearly all heterogenic crosses show a duality of parents. It may be that when the paternal influence is largely suppressed there is less disharmony in development and therefore a better chance for the hybrid to go through the critical periods of development without suffering from the discord between opposed parental tendencies. Even in the larvae, however, that are phenotypically pure or nearly pure maternal, there is doubtless carried latent or recessive in the germ plasm the full complement of paternal inheritance factors, that would segregate out in the following generation, if it were possible to interbreed the F_1 hybrids. *We are therefore not dealing with a case of parthenogenetic development but with one involving a more or less complete dominance of the egg over the sperm.*

Hybridization and the occurrence of monsters

Very many of the types of monsters that have been described in experimental and embryological literature occur with great frequency in hybrid strains. Cyclopic, uniophthalmic and anophthalmic monsters are of frequent occurrence. All grades of spina bifida and dicephalia occur. Double and triple tails are common as are headless and tailless forms; but none of these characters are to be attributed to the specific influence of the male parent. They are rather generalized pathological conditions due to mechanical or chemical retarding agents some of

which may result from a disharmonious interaction of foreign germ plasms. Some conditions however are strikingly specific for certain crosses. Perhaps the most pronounced case of this sort is that seen in the cross *Fundulus heteroclitus* ♀ × *Poronotus triacanthus* ♂. Here the embryos that developed most successfully are all narrow-headed. In rare cases the head may differentiate rather extensively but it always remains much too small and narrow for the body. This situation suggests some specific influence of the male cell in embryo formation. Again, to cite an opposite case, in the cross *Apeltes quadracus* ♀ × *Menidia beryllina cerea* ♂ there occurred very peculiar specific deformities. The head developed on one side of the yolk and tail at a distance on the other. This condition occurred in nearly all advanced embryos. At first I thought I had a case of polyembryony but found later that the condition is due to the fact that the mid-region of the body fails to develop, leaving the two extremities separate. This again indicates some specific influence of the male cell in embryo formation. Many other similar cases are cited in connection with the various crosses which tend to show that *abnormalities in particular crosses may be more or less specific and not merely general pathological conditions.*

Developmental blocks in heterogenic crosses

It seems to be generally true that the developmental rate of heterogenic crosses is slower than that of the pure bred maternal species. The retarding influence of the foreign sperm manifests itself during early cleavage in some crosses, during late cleavage in others, and not until gastrulation in still others. Appellöf calls attention to a well defined early retardation in the cross between *Spinachia* ♀ × *Gasterosteus* ♂, in which case the hybrid eggs were in the 4-cell stage when the pure bred *Spinachia* eggs were in 8-cell stages or farther. Moenkhaus cites a series of cases of early retardation ranging from early to late cleavage. In the cross *Tautogolabrus* ♀ × *F. heteroclitus* ♂ the hybrids are in 8-cell stages when the pure-bred *Tautogolabrus* are entering upon the 16-cell stage. In the cross *Gas-*

terosteus ♀ × *F. heteroclitus* ♂ the hybrid showed a less marked spread of the late segmentation disc than did the pure-bred embryos. Many other cases are cited that show evident retardation during gastrulation.

As a rule the earlier the retardation, the more pronounced the developmental block. Hybrids in which the retardation occurs during early cleavages are apt to disintegrate during late cleavage. Those that show no retardation until gastrulation is well under way, are apt to show a considerable degree of developmental success. The probable cause of early retardation is abnormal cleavage. The Hertwigs and Miss Morris show clearly that decidedly irregular cleavages occur in hybrid blastodiscs. This disruption of the normal process could not but result in retardation. It is also probable that later retardation is based on later irregularities in cleavages, for, in many of the crosses described in this paper, it was noted that the cell structure of certain regions is abnormally coarse, the cells being of uneven size and many of them of giant size. Such a condition indicates some abnormality in the cleavage mechanism.

It is remarkable, as Appellöf first pointed out, that *the most frequent stopping place in the development of heterogenic hybrids is at the end of the cleavage period, or just before the onset of gastrulation.* Since gastrulation is essentially a phenomenon of differentiation it is not surprising that we should find so many heterogenic crosses blocked at this point. For though mere cell multiplication or cleavage might proceed more or less successfully in spite of the irregular distribution of the chromatin, tissue differentiation cannot go forward under such conditions.

The next developmental block is that of embryo formation. Many crosses that enter upon early germ-ring stages without exhibiting any abnormalities come to a stop during middle and late germ-ring stages. It would appear that in such crosses the embryonic axis does not form signifying that concrescence of the two halves of the germ-ring fails to occur; hence the advance of the germ-ring is stopped at various points from one-fourth to three-fourths of the way around the yolk. Sometimes concrescence begins but soon stops. Again an embryonic axis is established but the

blastopore remains as an oval or slit-like opening. In such cases one of several things may happen. Either the germ-ring retreats to the animal pole of the egg and gathers into a lump-like embryonic mass that may form pigment cells and assume a vague likeness to a short embryo; or a flat embryonic shield with two rings of germ band enclosing an elongated blastopore may continue to live and differentiate regionally for some time; or advanced bodyless heads may develop and appear to end abruptly against a flat sheet of cells derived from the germ-band; or, finally, advanced embryos with well developed heads may occur at a distance from the posterior parts of the body separated from the latter by a flat germ-band region. All of these abnormalities are to be attributed to various interruptions of the process of concrescence. The beginning of gastrulation is the most general block in hybrid development but concrescence is scarcely less important. The cause of the latter block is not known, but is probably associated with the general retardation of development. The less energetic overgrowth of the yolk by the blastoderm is doubtless accompanied by a lowered fusion energy so that the two halves of the germ-ring do not so strongly attract each other. Many types of hybrid have no difficulty with gastrulation until the very last stage of blastopore closure. Such embryos develop for the most part normally, but show various defects of the tail region, where the metabolic rate is lowest, such as short tail or bent and spiral forms.

As a rule, hybrids that successfully weather the vicissitudes of gastrulation and are normal after the closure of the blastopore meet no distinct developmental block until the time comes for circulation to be established. It is very common for certain types of hybrids to go forward to advanced stages in which head and body are normally differentiated, in which the heart is formed and pulsating, but in which blood fails to develop and thus circulation is not established. The lack of a circulation at certain critical stages hinders, though it does not necessarily prevent, yolk assimilation, and various well defined organic disturbances result. The most frequent consequence of a failure to establish a circulation is seen in those types of hybrids in which the heart

is stretched out across the yolk in an enlarged pericardium. The heart beats but carries no blood. In other cases blood is formed but in such small amounts that symptoms of anemia soon appear and death ensues. If a full circulation is established the embryo has a good chance to hatch and to live as a normal larva but many cases have been cited in which embryos with circulation apparently normal have failed to hatch. It may be that in these cases the circulation was established too late in the developmental history and that it is not possible for the belated embryo to regain the lost ground.

It is quite common to find certain types of embryos that die shortly after hatching. As yet I have been unable to understand the low viability of these types. There are, however, here described many hybrid forms that hatch and seem to be normally viable, and I see no reason to doubt that these could be reared to advanced stages.

Practically all the well defined abnormalities and monsters of various sorts are, I believe, the result of a retardation of certain processes, chief among which is the process of concrescence. Cyclopia is doubtless, as Stockard has shown, due to a failure to separate on the part of the median ventral optic primordia of the brain floor. Double headed monsters are a result of a failure to conresce of the anterior germ-band, and spina bifida, double tail and similar abnormalities are likewise results of incomplete concrescence.

It is generally the case for heterogenic hybrids that in any strain some individuals surmount the developmental blocks that effectively stop others. In nearly all of the crosses described it has been noted that a considerable per cent of the hybrids cease to develop at the end of cleavage or during gastrulation, some may be blocked at the point of blood formation and others go on to hatching. What explanation can be offered for such a wide variation among embryos of common parentage? This same inquiry was made by Appellöf many years ago and the answer was deferred by him until such time as he should be able to conduct a more extensive series of researches on Teleost hybrids. I have what seems to me to be an adequate

mass of data bearing on this question but am still unable to give any satisfactory solution of the problem. It may, however, be suggested that the differences in success may be due to physiological differences in the condition of the egg and sperm, for it is probable that in any large number of germ-cells forcibly stripped from males or females some of each kind will be in better condition for development than others.

All the developmental blocks herewith cited are looked upon as developmental crises during which embryos are especially susceptible to disturbances introduced by a lowered metabolic rhythm. These crises probably represent the apices of curves of morphogenetic activity when the metabolic rhythm is at its height. The normal changes associated with these crises occur successfully only when the tonus of the rhythm is sustained at its proper level. A lowering of the tonus produces abnormalities. Any processes requiring less developmental pressure or a lower tonus go on irrespective of these blocks as we shall see in the next section.

Auto-differentiation and de-differentiation

It is not without interest in connection with these hybrid experiments to note certain phenomena of embryonic differentiation and de-differentiation, which are not specifically hybridization effects, but are merely the secondary result of abnormal conditions brought on through the disharmony of foreign germ plasms. In many of the experiments attention was called to the high degree of independence exhibited by certain types of cells and tissues. Chromatophores, for example, which normally appear only after considerable embryonic differentiation has occurred, develop and go to the definitive stages even when no other tissues may be differentiated. In those cases where the germ-ring retreats and forms a lump of tissue at the animal pole it often happens that such a formless embryonic mass becomes pigmented with the characteristic chromatophores of the species. Chromatophores can usually be relied upon to go their course and reach a definitive condition even when em-

bryonic differentiation stops at an early period. This result is taken to mean that chromatophores belong to a type of cells that are remarkably independent and are not closely correlated with the more highly organized tissues. They wander about and locate themselves in places of advantage. If no circulation is established they creep upon the beating heart, which never happens in normal embryos. When blood vessels form but no blood is developed chromatophores still gather about the blood vessels. This formation of vessels independent of blood is another illustration of tissue independence; but the most striking case of this sort is met with in those forms that develop a heart that continues to function even without a blood content. As I have noted repeatedly above, hearts of this sort are from the very first stretched out over the yolk so that they are under considerable tension. Yet they undergo the same flexures as when they are crowded within a restricted pericardium. It is commonly believed that the heart flexures are the result of a reaction of the heart to a restricted space, but when a stretched heart develops the same flexures this explanation becomes quite inadequate. It was noted also that hearts occasionally become entirely separated from the body of the embryo but, in spite of this isolation, they beat rhythmically, indicating that the heart itself is the seat of its own automaticity. It is in fact highly probable that such tissue is without ganglion cells and that the heart rhythm is therefore myogenic. Again it is often noted that even though the body may fail to cut itself off from the yolk, fins may develop out on the blastoderm that is still spread over the yolk. Such limb-buds may differentiate until they are capable of independent movement.

All these phenomena impress one with the idea that the embryo is less of a unit than we had supposed, that many semi-independent processes are at work to produce a harmonious end result and that one process may frequently be inhibited without interfering seriously with the others. It also appears that the particular fate of a region is determined rather early and that radically changed environmental factors fail materially to alter the end result.

While differentiation of certain parts goes on without hindrance other parts may be de-differentiating. Many cases are noted in which, without the general death of the embryo, certain regions that have reached an advanced stage of differentiation cease to develop and begin to lose their definition. This is seen most distinctly in connection with the head and particularly with the eyes. In some crosses the eyes and other special organs of the head develop normally to an advanced stage and then begin to grow vague. This appears to mean that the tissues are returning to a generalized state. They are not dead as is evident by the retention of their transparency, for dead tissue becomes more or less opaque. Heads diminish in size; pigment on the eyes migrates to other regions and the details of eye structure are lost. Such de-differentiated embryos may live for weeks and may continue to develop structures at a distance from the head end. In some cases de-differentiation involves only the most anterior regions of the head including the eyes. When the eyes are de-differentiated the otic vesicles become the dominant regions of the head and grow to unusual size. This would appear to mean that the eyes are the controlling elements of the head and that when they disappear the control or dominance passes back to the next elements, the otic vesicles. The student of developmental mechanics would find much to interest him in these cases and would probably discover many others equally significant.

SUMMARY

1. Ninety-three crosses among fourteen species of Woods Hole Teleosts were made and of these seventy-eight are heterogenic and are here dealt with. The homogenic crosses are to be treated separately.
2. *Heterogenic* crosses are those between different orders or families; *homogenic* crosses are between different genera of a family or between different species of a genus.
3. In seventeen heterogenic crosses some embryos established a circulation and in sixteen larvae were hatched.
4. There is as much developmental success among the heterogenic as among some homogenic crosses. In general there

seems to be no primary correlation between the degree of success in development and the nearness of relationship of the species crossed.

5. The factors determining the relative success in development of hybrids are associated with the amount, density and specific composition of the yolk, and with hardness of the egg.

6. The factor of rate of development appears to be a mere secondary factor in the success of hybrids as species with a slow development cross readily with rapidly developing species.

7. The differences in developmental success of reciprocal crosses are due to the same factors that lie at the basis of hybrid success in general. The hybrid from the species of egg that is more adaptable to hybridization will, irrespective of phylogenetic relationship, develop more successfully than the reciprocal cross from the less adaptable species of egg.

8. Many cases are cited in which definite paternal characters are inherited by heterogenic hybrids, which goes to disprove Loeb's conclusion that heterogenic hybrids are pure maternal and that the sperm plays only the rôle of initiating development, without having any share in embryo formation or heredity.

9. In all the crosses in which some embryos develop to hatching the most successful of them are predominantly maternal; but less successful hybrids show both maternal and paternal influence in varying degrees. The nearly pure maternal larvae are thought to be merely phenotypically so, for it is likely that this germ-plasm carries its full quota of paternal inheritance factors.

10. All of the well known types of monsters occur in hybrid strains. These abnormal conditions are in some cases specific for certain crosses and others are of a more generalized character occurring in many crosses. All of the abnormal types are capable of being interpreted as retardation products and therefore as due to the slowing down of development through the disharmonious interaction of foreign germ plasmas.

11. The principal developmental blocks noted in heterogenic hybrids occur in connection with the following crises: gastrulation, coelomic differentiation, head differentiation, the establishment of a cir-

ulation. These crises probably represent the apices of curves of morphogenetic activity, when the metabolic rhythm is at its height. A lowering of the rate of metabolism affects these processes more profoundly than it would processes associated with a low developmental tonus.

12. Many cases of independently differentiating tissues are cited, chief among which are chromatophores, heart, fins, etc. These structures continue to develop when isolated from their normal environment. De-differentiation of the more highly differentiated parts occurs readily even while the less differentiated parts continue to grow. General death occurs slowly and some tissues are particularly resistant. Chromatophores go on to their definitive state even when the tissues of the embryonic body are reduced to a generalized mass of cells.

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THE REACTIONS OF THE MELANOPHORES OF AMBLYSTOMA LARVAE

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INTRODUCTION

In a recent publication dealing with the phototactic responses of the larvae of *Amblystoma punctatum* (Laurens '14 a) a brief mention was made of the intensity of pigmentation of normal and eyeless individuals in diffuse light and in darkness. It was there stated that the coloration of normal and eyeless larvae was different, although they were kept under identical conditions.

The results were briefly as follows: Normal larvae placed in diffuse light on an indifferent background were pale, while normal larvae placed in darkness were dark. Eyeless larvae placed under these conditions showed exactly the opposite conditions of coloration, in that in diffuse light the larvae were dark, in darkness they were pale. In addition to these responses to light and darkness certain others to background were also mentioned. Normal larvae placed in diffuse light on a white background were pale while those placed on a black background were very dark. The eyeless larvae show no responses to background.

The observations which led to these results were made on larvae that were being used to test their phototactic responses. Groups of ten larvae were placed in darkness or in light, on white or on black backgrounds, where they were allowed to remain for varying lengths of time, and were observed only at the time when the phototactic tests were made and usually only with the naked eye, the general appearance—pale or dark—being taken as the criterion of the coloration.

The results that have just been briefly outlined are in complete agreement with those of Babak ('10^a, '10 b, '12, and '13) for the Axolotl larvae (*A. mexicanum* cope, *A. tigrinum* Laurenti). Pernitzsch ('14) in a recent study of the chromatophores of these larvae has also found that keeping the larvae in the dark causes them to become dark, while keeping them in the light (on white backgrounds) causes them to become pale.

The experiments, with the results of which, the present paper has to deal were instigated by a desire to analyse more closely the differences in the coloration of normal and eyeless larvae under the same conditions. Babak has given a rather lengthy theoretical consideration of these differences in the Axolotl and has proposed some interesting hypotheses to explain them. It seemed to me desirable to attempt to find out something more about the actual behavior of the melanophores themselves and to study not only the effects of light but of various other physiological stimuli as well, viz., temperature, various solutions of salts and drugs, and electric currents, both induced and constant; further to extend this study to larvae that had been deprived

of their eyes and to those in which the nervous system had been partially or totally destroyed.

With this end in view experiments were started in the early spring months of 1914 on normal and eyeless larvae, exposing them to the conditions of light, background and darkness mentioned above. The methods of blinding and keeping the larvae were the same as those described in my earlier paper and consisted in removing the optic vesicles from embryos between 4 and 5 mm. long in which the tail bud is just appearing, and in which no nerve fibers have become differentiated. The advantages of operating at this stage over the method of removing the eyes later, need only be referred to. The larvae, both normal and eyeless, were first placed in small stender dishes, from which they were later transferred to battery jars and finally to large stender dishes of 18 cm. diameter and 8 cm. deep which held about 1200 cc. of water.

As every one knows, the literature dealing with the chromatophores, both morphological and physiological, is enormous. Since in most of the many recent publications the previous literature has been reviewed and brought up to date it shall not be an object of the present contribution to do so again, but merely to call attention to those papers which seem to have a particular bearing on the results here detailed.

REACTIONS TO LIGHT

In these experiments both daylight and artificial light were used, the latter being furnished by a Nernst glower and by a small arc lamp. The backgrounds used were three in number: (1) an 'indifferent' background, either grayish in tone, obtained by placing the dishes on a black wooden tabletop, or on a gray soapstone one; or brownish-yellowish, obtained by placing the dishes on a tabletop of such a color; (2) a black background, obtained by painting the bottoms of the dishes with dull black japalac; and (3) a white one, obtained by covering the sides and bottoms of the dishes with white paper.

1. Reactions of the melanophores of seeing and eyeless larvae

The reactions of the normal and eyeless larvae may best be considered together. A large number of observations were made upon individuals which were grouped into various series. In order to show how extensive these were and how thorough an analysis of these reactions has been attempted, the following outline is given.

a. Outline of experiments. 1. Normal larvae in bright diffuse daylight, white background compared with black.

2. Normal larvae in bright diffuse daylight on indifferent, white and black backgrounds compared with normal larvae in darkness.

3. Eyeless larvae in bright diffuse daylight, white background compared with black.

4. Eyeless larvae in bright diffuse daylight, on indifferent, white and black backgrounds compared with eyeless larvae in darkness.

5. Normal larvae compared with eyeless larvae: (a) normal larvae on a white background compared with eyeless larvae on a white background; (b) normal larvae on a black background compared with eyeless larvae on a black background; (c) normal larvae on an indifferent background compared with eyeless larvae on an indifferent background; (d) normal larvae in darkness compared with eyeless larvae in darkness.

Under group 5 the conditions found under the other four groups are duplicated. This was done intentionally for the sake of obtaining direct comparisons and increasing the number of individuals observed. As will be seen later, some of the comparisons were useless, e.g., those of eyeless individuals on the various backgrounds, but they furnished a larger number of eyeless individuals for observation. Three larvae were used under each of the conditions and this first series therefore consisted of 60 individuals. In all ten series of larvae were used, five for *A. punctatum* and five for *A. opacum*. All of the series did not include all of the comparisons given in the outline, but

in each series of comparisons made three larvae were used for each condition.

In all but one of the five series of *A. opacum* larvae the animals had the use of their eyes. It was desirable to carry out experiments on these larvae from which the power of sight had been taken away. On the other hand, operations upon the eyes at this stage of development (the larvae were all between 35 and 55 mm. long) were considered as too serious. Since operations upon the eyes of the *opacum* larvae were out of the question some method of painting them over was decided upon as the best way to deprive these larvae of the use of their eyes. Two substances proved themselves practicable for this purpose. The first, and better, was a solution of celloidin in ether to which lampblack had been added. The second was a fairly thick solution of shellac in alcohol. The method of painting over the eyes was briefly as follows: A larva was first placed in a 0.02 per cent solution of chloretone until it had almost lost all power of movement, it was then transferred by means of a large section lifter to a damp cloth, and a piece of lens paper thoroughly soaked in water tucked around it so that the gills were covered. The head was then dried as much as possible by means of filter paper and with a small camel's hair brush a fine drop of either celloidin or shellac was applied to each eye over which it formed a low mound. After waiting a few seconds to allow the drop to dry and to become fairly firm, the larva was placed in a large stender dish filled with water, where it soon recovered. It was found necessary not to keep the larvae too long in the chloretone solution or in the air, else they soon died. Five larvae were thus successfully blindfolded and observations made on them from April 11 until May 16, when three of them were preserved.

The blinds of celloidin or shellac could not be made to stay permanently over the eyes, and after a week or so invariably came off. Two larvae to which this had happened had been again blindfolded and preparations were being made to re-cover the eyes of a third, when it was noticed that the corneas and surrounding epidermis were very thick and opaque. Examined

under a binocular microscope it was seen that the corneas in addition to being thick and opaque had become invaded by a large number of pigment cells which were expanded. This condition presented substantially a blindfolded one; that it really was such will be apparent from the results of the observations carried out upon these larvae.

Several of the opacum larvae metamorphosed during the course of the experiments. The observations were continued upon these young adults since it was soon noted that their pigment cells responded in the same way as those of the larvae, only considerably more slowly and less extensively.

The conditions of the melanophores themselves and not the general appearance of the larvae must be emphasized. The larvae of both *A. punctatum* and opacum show the black and white races that have been described by Haecker ('07, '12 a, and '12 b), and more recently referred to by Pernitzsch ('13), for the Axolotl. As these observers have shown, the white race is not a true albino, since there is always some black and yellow pigment. The 'Scheck' or 'Metamerscheck' described and figured by Haecker and also by Goldschmidt ('13) was not observed among the larvae of *punctatum* and opacum. The occurrence of these two races of *Amblystoma* can cause a great deal of confusion and mistaken judgment if the general appearance—dark or pale—of the larvae alone is considered in describing the general condition of coloration or pigmentation of the larvae under the different conditions of illumination, etc. A 'white' larva with expanded melanophores is not unlike a 'black' one with contracted pigment cells. Under the binocular microscope the individual pigment cells are themselves observed and no mistake as to whether they are expanded or contracted is possible. In addition, when melanophores or pigment cells are spoken of, it is the sub-epidermal pigment cell that is always referred to, unless otherwise stated. The melanophores in the epidermis, which are of different form from those in the sub-epidermis, are more irregular and variable in their responses than are the sub-epidermal pigment cells, and very often, particularly in the larvae that have been under a certain condition for some

time, the one form of pigment cell will be contracted, the other expanded, and vice versa. The xanthophores are, as far as could be made out, always expanded under all conditions of illumination and darkness.

b. The state of the melanophores under long continued conditions of illumination and darkness. The state of the pigment cells of larvae that have been kept under a particular condition for some time can be briefly stated. Normal seeing larvae that have been kept in bright diffuse daylight on a white background always show a contracted state of the sub-epidermal melanophores. The epidermal melanophores may, however, be contracted or expanded and a larva may indeed appear 'dark' on a white background if the epidermal melanophores are numerous, although the sub-epidermal melanophores are maximally contracted. Normal larvae that have been kept in bright diffuse daylight on a black background show a maximally expanded condition of all of the melanophores, epidermal usually as well as sub-epidermal.

In normal larvae that have been kept for some time in bright diffuse daylight on an indifferent background the melanophores are in a condition of, what for convenience has been called, $\frac{1}{8}$ to $\frac{1}{4}$ expansion, and are never fully contracted to dots or points. Such larvae have the same general appearance as larvae that have been kept on a white background and are indeed 'pale.' In normal larvae that have been kept in absolute darkness for a considerable time the melanophores are invariably expanded. They are, however, never, or very seldom, expanded to their utmost, and are in a state of expansion that has for convenience of description been called a $\frac{3}{4}$ to $\frac{7}{8}$ expansion.

The conditions of the melanophores that have been described for the larvae kept in the light do not refer to larvae that are kept in aquaria containing water plants and with a bottom of dark mud or sand. Here the conditions of illumination are naturally quite different from those in aquaria with clear water, free from plants and with clean bottoms. In aquaria with plants, etc., the condition of the melanophores is that described for the larvae on a black background, and in such an aquarium the background may be said to be really a black one, since most of the

light is absorbed. In addition the plants produce shadows, causing dark places, where the larvae are most likely to be found.

Eyeless larvae do not respond to differences in background. In general in all eyeless larvae kept in the light the melanophores are fully expanded, both epidermal and sub-epidermal. In eyeless larvae kept in the dark all the sub-epidermal melanophores are maximally contracted, while the epidermal pigment cells may or may not be. These larvae are usually very pale and transparent. The xanthophores are, as usual, expanded and can be sometimes seen with great distinctness in the anterior portions of the body on the head and trunk as well as on the tail, where they are usually only to be seen.

TABLE 1

State of the melanophores after long continued illumination and darkness

BACKGROUNDS	LIGHT			DARKNESS
	White	Black	Indifferent	
Normal	contracted	expanded	contracted ($\frac{1}{8}$ - $\frac{1}{4}$ exp.)	expanded ($\frac{3}{4}$ - $\frac{7}{8}$)
Eyeless	expanded	expanded	expanded	contracted

The state of the melanophores under all of the conditions for both normal and eyeless larvae have now been described, and are given in table 1. Summarized they are as follows: the melanophores of normal larvae on a white background are contracted, on a black background expanded, in bright diffuse daylight (on an indifferent background) contracted ($\frac{1}{8}$ to $\frac{1}{4}$ expansion), and in darkness expanded, ($\frac{3}{4}$ to $\frac{7}{8}$ expansion). The melanophores of eyeless larvae in bright diffuse daylight are expanded, in darkness contracted. In general, the eyeless larvae are darker and paler than the seeing larvae owing to the incomplete expansion and contraction of the melanophores of the larvae with eyes. The condition of the melanophores of normal and eyeless larvae are, as was previously stated, exactly opposite under identical conditions of illumination and darkness. But since these are the conditions which have already been described

(Babak and Laurens) the question may be raised as to why have they again been described. The answer to this question is that, although the conditions are as they have been described when the seeing and eyeless larvae are allowed to remain for some time under the particular conditions, they do not represent the primary responses of the melanophores, which are identical in the two kinds of larvae.

c. *The reactions of the melanophores to changes in light and darkness.* The general course of procedure in these experiments was to place the members of the various series of larvae in groups of three under the different conditions of illumination, etc., and

TABLE 2

The reactions of the melanophores of normal and eyeless larvae to light and darkness

	LIGHT INDIFFERENT BACKGROUND	DARKNESS
Normal		
I. reaction	expansion ($\frac{3}{8}$) ($1\frac{1}{2}$ -2 hrs.)	contraction (2-3 hrs.)
II. reaction	contraction ($\frac{1}{8}$ - $\frac{1}{4}$ exp.) (3-5 days)	expansion ($\frac{3}{4}$ - $\frac{7}{8}$) (5 days or more)
Eyeless		
I. reaction	expansion (2-3 hrs.)	contraction (4-5 hrs.)

after allowing them to remain for varying lengths of time to transfer them to some other and to observe the changes in the melanophores caused by the new condition. In describing the results it will be best to take up the consideration of the reactions of the melanophores of the normal larvae for each possible change, and to begin with the larvae that are on the indifferent backgrounds in bright diffuse daylight (table 2). The melanophores of such larvae, it will be remembered, are contracted ($\frac{1}{8}$ to $\frac{1}{4}$ expansion). When they are transferred to darkness the response of the melanophores is to contract still further until they are all mere dots, so that the larvae really become 'pale.' This response is completed in from two to three hours. If, after the melanophores have become completely contracted, the larvae are carried back to the light, in from $1\frac{1}{2}$ to 2 hours the melanophores are all expanded ($\frac{7}{8}$ expansion). This is not, however,

the condition in which the melanophores were when they were first taken from the light, for then they were in a contracted condition ($\frac{1}{8}$ to $\frac{1}{4}$ expansion), which, as has been shown above, is the typical condition of the melanophores that have been kept for some time in the light. Herein lies the difference in the results. If the larvae are allowed to remain in the light, undisturbed for from three to five days this contracted condition of the melanophores is brought about. In other words, the light which at first caused the melanophores to expand, when allowed to act for some time causes them secondarily to contract.¹

It has just been said that when larvae are transferred from bright diffuse daylight to darkness that the melanophores still further contract until they are all mere dots. But the characteristic condition of the melanophores of larvae that have been kept in darkness has already been described as one of expansion ($\frac{3}{4}$ to $\frac{7}{8}$; p. 583). This expanded condition of the melanophores in darkness is indeed the typical one and is always found in larvae that have been allowed to remain in darkness for more than five days.

When larvae that have been kept so long in the dark (more than five days) that the melanophores have all secondarily expanded, are placed in bright diffuse daylight, the melanophores give no response, but simply remain expanded. If the larvae are now allowed to remain in the light, the melanophores again after from three to five days all contract ($\frac{1}{8}$ to $\frac{1}{4}$ expansion). If, however, the larvae are not left in the light but are transferred back to darkness, after an exposure of an hour or so to light, the melanophores contract in the average time of two to three hours.

Eyeless larvae do not show these 'secondary' responses to continued illumination and darkness. When eyeless larvae are taken from the light to darkness, the melanophores which have

¹ Most of the larvae when transferred to the light were not constantly illuminated, so that at night the melanophores contracted and the next morning re-expanded. Such larvae, however, show no constant differences in the length of time necessary for the secondary contractions to come about from those that are illuminated at night by an electric light, which is of sufficient intensity to cause the melanophores to expand when the larve are removed from darkness.

been maximally expanded soon contract until they are mere dots, the response being completed in from four to five hours, the time necessary being therefore somewhat longer than in normal seeing larvae. But no matter how long these blinded larvae are kept in darkness the melanophores never expand again. When these larvae are returned to the light the melanophores expand in from two to three hours, and remain so as long as they are kept illuminated.

The normal seeing and eyeless larvae of *A. punctatum* therefore give reactions to light and darkness which are identical; and only secondarily are the states of the melanophores exactly opposite under identical conditions.

There still remain to be considered the changes that take place in the melanophores when normal larvae are transferred from one background to another. Also those that take place when such larvae are transferred from these backgrounds to darkness and back again.

When larvae that have been kept in bright diffuse daylight over an indifferent background, until the melanophores have secondarily contracted ($\frac{1}{8}$ to $\frac{1}{4}$ expansion), are transferred to a white background the melanophores in a very short time completely contract, the response being completed in from 20 to 30 minutes (table 3). If they are now transferred back to the in-

TABLE 3

The reactions of the melanophores of normal larvae to a change in background

BACKGROUND	INDIFFERENT	WHITE
reaction	contraction ($\frac{1}{8}$ - $\frac{1}{4}$ exp.) (Time = ?)	complete contraction (20-30 min.)
BACKGROUND	INDIFFERENT	BLACK
reaction	contraction ($\frac{1}{8}$ - $\frac{1}{4}$ exp.) (1-2 days)	complete expansion (30 min.-1 hr.)
BACKGROUND	WHITE	BLACK
reaction	complete contraction (3-4 hrs.)	expansion ($\frac{7}{8}$) (1 hr.)

different background it is very difficult to say just how long it is before the melanophores become again $\frac{1}{8}$ to $\frac{1}{4}$ expanded, but it is certain that under such conditions the melanophores never remain for very long completely contracted. Sometimes they expand more than $\frac{1}{8}$ to $\frac{1}{4}$, but in these cases they soon return to this condition.

When normal seeing larvae that have been kept on an indifferent background are transferred to a black one, the melanophores begin to expand very quickly and in thirty minutes to an hour the pigment cells are all maximally expanded. When they are transferred back to the indifferent background it usually takes only one to two days (instead of three to five) for the melanophores to again contract to the typical condition of $\frac{1}{8}$ to $\frac{1}{4}$ expansion.

When larvae that have been kept on a white background in bright diffuse daylight, where the melanophores are all completely contracted, are placed on a black one, in less than fifteen minutes the black pigment cells begin to expand and in an hour they are all $\frac{7}{8}$ expanded. If they are now transferred back to the white background the melanophores take from three to four hours to contract. When the larvae are kept for considerable lengths of time on the white or black backgrounds, and this is true also of larvae that have been kept for some time in the dark, the reaction time of the melanophores is very much increased. Bauer ('10) has noted the same thing in flat fishes (Pleuronectids) and Babak also refers to this as taking place in the Axolotl. Bauer thinks that this lengthening of the time in which the response comes about is due to the tonus of the pigment cell gradually becoming independent of the impulses set up by the stimulation of the eyes, and to the development of a relative automatic condition (*Automatie*) of this tonus.

If normal larvae that have been kept on a white background are transferred to darkness the melanophores show no response (table 4). This seems strange, but since the melanophores are already in the condition that they assume when transferred to darkness there is nothing for them to do but to remain in that condition. The secondary response of expansion to darkness

comes about in the usual time (5 days or more). When these larvae, before the melanophores have secondarily responded in the dark, are transferred back to bright diffuse daylight over a white background, the black pigment cells invariably expand, that is light exerts its normal effect of causing the melanophores to expand although the larvae are on a white background. This response of expansion comes about in the usual average time of $1\frac{1}{2}$ to 2 hours. Only after some three to four hours after this do the melanophores contract in response to the white background. If these larvae, however, are not transferred back to the light

TABLE 4

The reactions of the melanophores of normal larvae to backgrounds and to darkness

WHITE BACKGROUND		DARKNESS
I. reaction	expansion ($\frac{7}{8}$) ($1\frac{1}{2}$ -2 hrs.)	none, remain contracted
II. reaction	contraction (3-4 hrs.)	expansion ($\frac{3}{4}$ - $\frac{7}{8}$) (5 days or more)
BLACK BACKGROUND		DARKNESS
I. reaction	expansion ($1\frac{1}{2}$ -2 hrs.)	contraction (7-8 hrs.)
II. reaction	contraction (3-4 hrs.)	expansion ($\frac{3}{4}$ - $\frac{7}{8}$) (5 days or more)

until after the secondary response of contraction to darkness has taken place, then they remain expanded over the white backgrounds only for three to four hours and then contract.

When normal larvae that have been kept on a black background, so that all the melanophores are $\frac{7}{8}$ expanded, are placed in darkness (table 4), it takes somewhat longer for the black pigment cells to contract than it does in the case of larvae that are taken from indifferent backgrounds where the melanophores are still in the primary expanded condition. In this case it is some seven to eight hours before the melanophores will be found to be contracted. The secondary response, of course, comes about in the same length of time, (5 days or more), as in the other cases. When larvae are transferred from darkness to black backgrounds in bright diffuse daylight the melanophores expand as

usual in $1\frac{1}{2}$ to 2 hours. Light then exerts its effect of causing the melanophores to expand no matter what the background is, and just as the melanophores of larvae over an indifferent background secondarily contract so also do they, only secondarily, contract in larvae on a white background.

The eyeless larvae not only do not respond to differences in background, but when kept on various backgrounds show no differences in the lengths of time requisite for the melanophores to contract when the larvae are transferred to darkness.

TABLE 5

The reactions of the melanophores of blindfolded larvae to light and darkness

BACKGROUNDS	LIGHT			DARKNESS
	White	Black	Indifferent	
I. reaction	expansion (2-3 hrs.)	expansion (2-3 hrs.)	expansion (2-3 hrs.)	contraction (4-5 hrs.)
II. reaction	none	none	none	expansion ($\frac{3}{4}$ - $\frac{1}{2}$) (5 days or more)

Babak suggests that perhaps the melanophores of Axolotl larvae that have been blindfolded by painting over the eyes, the retinae not being destroyed, would not respond to light and darkness as do those of larvae which have been deprived of their eyes. The opacum larvae whose eyes were painted over in the manner described above afford an opportunity of putting this suggestion of Babak's to the test. It will be remembered that the thickened and opaque condition of the cornea was believed to be substantially a blindfold. None of these larvae showed any of the responses of normal seeing larvae to background, and over indifferent, black and white backgrounds the melanophores remained expanded as in eyeless individuals (table 5). When these blindfolded larvae are transferred to darkness the melanophores contract, which is characteristic of eyeless as well as of normal seeing larvae. But in these blindfolded individuals the secondary response to darkness takes place just as in normal seeing larvae, and after five or more days the melanophores will be

found to be expanded. The results obtained in the light leave no doubt that the eyes were really blindfolded, since the melanophores responded as in eyeless larvae.

2. Reactions of the melanophores of young adults

The reactions to light of the melanophores of young adults were substantially the same as those of the larvae. Sometime before the larvae of opacum metamorphose the body becomes covered with small grayish patches which under the binoculars can be seen to be caused by the development in the skin of a whitish pigment which becomes rapidly more abundant and disposed in rather definite patterns over the body, producing the so-called 'marbling' of these salamanders. At the same time the black pigment cells become more diffuse, the cell processes apparently anastomosing. At the same time that this whitish pigment is developing a much increased blood supply to the skin is noticed. Blood vessels, in which the moving capillaries can be seen, become thickly distributed over the sides and dorsum of the larvae, and the gills can be seen to begin to shrivel and gradually to disappear. The changes in the larvae of punctatum are practically identical, with the difference that the yellow pigment becomes aggregated to form the yellow spots characteristic of the species.

The responses of the melanophores of these young adults, although similar to those of the larvae, take much longer to come about and are not as complete. Larvae that metamorphosed under the different conditions were remarkably different in appearance. The opacum larvae on a white background were flesh colored with white splotches here and there, the melanophores being completely contracted. Those on a black background and in darkness were very dark with white splotches. The punctatum larvae showed similar conditions with yellow dots here and there. But when the larvae were interchanged, it took some time, particularly in those transferred to the white background, for the melanophores to respond, and there was a tendency for the melanophores to assume a middle condition. The

larvae that had metamorphosed on indifferent and white backgrounds showed more quickly than the others the changes in the melanophores when they were transferred to other conditions.

3. Experiments to determine the stage of development at which the melanophores first respond to light and darkness

The reactions to light of the melanophores have so far been described for the larvae in general. The melanophores of very young larvae, however, do not show the responses to light and darkness. The normal seeing and eyeless larvae are alike in coloration and the melanophores in all are expanded. It is not until a particular stage of development is reached that the melanophores acquire the ability to respond to the stimuli of light and darkness and to differences in background. Babak ('10 a, p. 112) has noted that the differences between seeing and blinded larvae are not to be observed in the very young axolotl larvae (17 mm. long) and refers this to the lack of development of the eye, or of the pigment controlling function of the retina. At this time, according to Babak the retina has probably also not yet acquired the capacity of receiving light stimuli or of making an image. Before this period the normal larvae possessing eyes show the same condition of pigmentation in darkness that the blinded do—the melanophores being all contracted. From these facts Babak argues that the pigment motor function of the retinae gradually develops after the irritability of the melanophores to direct stimulation has already appeared, and that in the developing retinae certain conditions are gradually attained, by means of which, through the central nervous system, they govern the movements of the chromatophores, and give to the skin its characteristic appearances in the light and in darkness.

A set of observations on punctatum larvae was started with the view of finding out at just what stage of development the melanophores of the larvae were able to respond to the stimuli of light, background and darkness. More particularly to determine (1) just when normal larvae first showed contracted melanophores when they were reared in bright diffuse daylight .

on white backgrounds, or when placed on them; (2) just when normal larvae first show the 'secondary' responses to light and darkness; and (3) just when normal and eyeless larvae first show contracted melanophores when they are reared in darkness.

Eycleshymer ('06) has shown that, when observed under the binoculars, the melanophores of *Necturus* larvae (11 to 12 mm. long) can first be seen to appear as fine dots lying deep down in the transparent connective tissue, that later (in larvae 15 to 16 mm. long), they reach the outermost surface of the dermis where they form an open meshwork. Eycleshymer has also studied the gradual development and outward migration of the pigment cells by means of sections. Wilson ('97) has shown that in *Amblystoma* larvae, the development of which have been stopped by placing them in 0.06 per cent NaCl and in Ringer solutions, that the pigment is formed *in situ* in the yolk cells themselves.

In larvae of *Amblystoma punctatum* the melanophores, as seen under the binoculars, first appear as fine dots which soon show a few processes and later take on the typical branched or radiating form. The black pigment cells are at first few and quite distinct from one another. Pernitzsch ('13) has recently given an account of the various forms of melanophores that can be distinguished in the skin of the axolotl, and most of these have been casually seen in the skin of *punctatum* and *opacum*.

In these observations the larvae were placed, in early stages of development, usually in the early neural fold stages, under the various conditions of light and darkness. The larvae reared in darkness showed no differences, either in the time when the melanophores were first discernible or in the number of melanophores, as compared with those reared in the light on indifferent backgrounds. Comparisons made between larvae reared from these stages in the light and then transferred to darkness, and those reared only in the dark, showed also no differences, not only in the above particulars, but also not in the time when the ability of the melanophores to respond to the various conditions first made its appearance.

The melanophores of larvae that are reared in the light on an indifferent background are all expanded until the larvae reach an average length of 17.7 mm. After this the melanophores are observed to be contracted ($\frac{1}{8}$ to $\frac{1}{4}$ expansion). The melanophores of larvae that have been reared on black backgrounds when transferred to indifferent backgrounds also do not contract until the larvae have reached this stage of development.

The melanophores of larvae that have been reared in the light on a white background do not contract until the larvae attain an average length of 16 mm., the extremes being 14.2 mm. and 18 mm. In those reared in the dark the melanophores do not contract until an average length of 13.3 mm. is reached, the extremes here being 12 mm. and 15 mm. The melanophores of larvae that have attained the average length of 18.4 mm. show the secondary response to darkness already developed. In larvae that have been reared in the dark and in those that have been reared in the light and then transferred to darkness at this stage, the melanophores in the usual length of time (5 days or more) expand. The larvae at this time are growing fairly rapidly, it has been computed from daily measurements that up to the time that the larvae begin to feed (16.7 mm. to 18 mm. long) growth in length takes place on the average at the rate of 0.5 mm. per day, and since this secondary response to darkness takes five days or more to come about these larvae have grown considerably during that time. But the melanophores of larvae taken from bright diffuse daylight at slightly earlier stages do not act any differently and it can be concluded that the retinae not having developed sufficiently (although the larvae are in darkness) the specific impulses are not set up in the retinal elements which bring about the secondary expansion of the melanophores in darkness, and that therefore the larvae do not show the melanophores expanded at any earlier stage of development. It is doubtful that the development of the primary responses of the melanophores has anything to do with the development of the nervous system and it is probably alone due to the development of the direct irritability of the melanophores themselves.

The melanophores of eyeless larvae that have been reared in darkness first show a contracted condition of the melanophores in larvae that have reached an average length of 13 mm. (the extremes being 12.2 mm. and 13.6 mm.). This is only slightly earlier than in normal seeing larvae and the difference is without significance.

It has just been noted that the melanophores of normal seeing larvae contract in 13.3 mm. larvae in the dark and that in 16 mm. larvae the retinae have so far developed that the melanophores contract when the larvae are on white backgrounds. In addition, that it is not until larvae have reached an average length of 18.4 mm. that the secondary response of the melanophores to darkness takes place, and that when reared in bright diffuse light on an indifferent background the larvae are of an average length of 17.7 mm. before the melanophores will secondarily contract. These facts point directly to the conclusions that the ability of the melanophores to respond to a direct stimulus—or to the lack of a stimulus (darkness)—is first developed; that after this the response to background through the eyes, and the secondary response to light, which are both dependent upon the retinae and the central nervous system, are developed, and that last, the secondary response to darkness makes its appearance, this last of course also being dependent upon the retinae and the central nervous system. Furthermore, it can be assumed that, since the retinae of 16 mm. larvae are so far developed that the larvae respond to differences in backgrounds, the retinae of these larvae are also affected when the larvae are kept on indifferent backgrounds. But as we have seen it takes from three to five days for the secondary response of the melanophores to light to take place and during this time the larvae have attained an average length of 17.7 mm.

4. The reactions of the melanophores in isolated pieces of skin

Small pieces of skin were removed under approximately aseptic conditions from the heads and dorsal surfaces of larvae in some of which the melanophores were expanded in others of which they were contracted. These pieces of skin were placed on ster-

ile cover slips in small drops of various fluids and inverted over sterile depression slides. The fluids in which the bits of skin were placed were various, and the following may be mentioned since they are the ones with which several experiments were carried out. Frog plasma 0.65 per cent sodium chloride, a Ringer solution (Clark), various normal or molecular solutions of sodium and potassium chloride, solutions of chloretone, atropin, atropin sulphate, and curare and finally tap and distilled water. A discussion of the effects, or lack of effects, of these various substances will be taken up later under the section devoted to the reactions of the melanophores to various solutions. They are mentioned here merely because they were also used in connection with these light experiments.

The results of exposing isolated bits of skin of *Amblystoma* larvae to daylight and darkness were entirely negative. Melanophores that were contracted when the skin was removed remained so whether placed in bright diffuse daylight, sunlight, or in darkness and the same is true of expanded melanophores. Bits of skin were removed in darkness, with just enough illumination to make the operation possible, from larvae in which the melanophores were expanded or contracted, with the same results. Since daylight did not bring about an expansion or contraction of the melanophores the light from a Nernst glower passed through a water cell and a lens, by means of which the rays were made parallel, was tried, but also with negative results. The bits of skin were exposed to the light by placing the slides on the stage of a compound microscope and reflecting the light upon them by means of the plane surface of the mirror. Also by placing the slides with the bits of skin directly in the path of the beam of parallel rays of light. The illumination was continued as long, in some cases, as eight hours, with no changes in the melanophores being apparent. That the melanophores were still alive at the end of that time and could still respond to stimuli was shown by stimulating them electrically, when the appropriate responses were to be obtained. It may be mentioned, in passing, that the bits of skin placed in frog plasma

lived as long as five days, responding at the end of that time to electrical stimulation.

From these experiments it might be said that the melanophores in isolated pieces of skin entirely deprived of the normal connections—blood and nervous—with the body, are not responsive to light, and but for later experiments it might be argued that the melanophores in skin isolated from the body show no ability of being stimulated directly by light. Spaeth ('13 b) in attempting to take photographs with arc-light illumination of the expanded melanophores in isolated *Fundulus* scales found, that before the necessary preparations were made, the melanophores were all contracted. When removed from the stage of the microscope the melanophores again expanded. When exposed to blue light these melanophores did not contract and Spaeth concluded that the contraction might be due to the ultra-violet rays of the arc-light. Experiments with ultra-violet light proved this to be the case, since it also caused the melanophores to contract. It seemed worth while to me to see whether these rays could be shown to have any effect on the melanophores of the isolated pieces of skin of *Amblystoma* larvae. Accordingly, bits of skin were illuminated by means of a small arc-lamp, (Leitz Lilliput, 4 to 5 amp., 110 volts) from which the condensing lens was removed. These pieces of skin were placed on a depression slide in a drop of salt solution and illuminated from above, the bit of skin being observed through the microscope. Illuminated in this way it was found that in from twenty to twenty-five minutes the melanophores which had previously been maximally expanded were completely contracted. In order to prevent the heat of the arc from having an effect of contraction a continuous blast of compressed air was blown over the slide. A thermometer placed on the stage never arose above 22°C. while, as will be shown later, a temperature of 38°C. was found necessary to bring about contraction.

In these arc-light illumination experiments the bits of skin in addition to being placed in salt solution were also immersed in drops of atropin, atropin sulphate and chloretone. These are

perhaps deserving of special mention. Hertel ('07) found that when the nerve endings in the skin of young *Loligo* were rendered insensitive by a 1 per cent solution of atropin that the contraction of the chromatophores in ultra-violet light took place just as before. He therefore concluded that the nerves were not necessary for the response which was brought about by direct stimulation of the pigment cells. Bits of the skin of *Amblystoma* larvae placed in 1 per cent and 2 per cent atropin also show the contraction of the melanophores when illuminated with the arc-light and in the same length of time as when placed in salt solution. Again, Spaeth ('13 b) found that a 1 per cent atropin sulphate solution causes the melanophores in isolated *Fundulus* scales to expand. This result has never been obtained with the melanophores of isolated bits of *Amblystoma* larva skin. When pieces of the skin are placed in drops of atropin sulphate, as highly concentrated as 4 per cent contractions of the melanophores still come about when the skin is illuminated with the arc-light. When, however, the bits of skin are placed in a 0.01 per cent solution of chloretone,—which, as we shall see later, brings about an expansion of contracted melanophores in living larvae as well as of those in isolated pieces of skin—and are illuminated with the arc-light, a contraction of the melanophores no longer takes place. When the solution of chloretone is reduced to 0.005 per cent contraction still takes place but not completely, and only after a longer illumination (three-quarters of an hour or more being necessary).

Several investigators have noted the responses of the melanophores to daylight and darkness in skin which has been isolated from the body. Hooker ('12) has described these responses in pieces of the skin of *R. fusca*. The melanophores, when suspended in drops of plasma, expanded in the light and contracted in darkness for a day, but after that showed the reverse responses. Ballowitz ('14) demonstrated that in the larvae of a fish (*Gobius minutus* and *G. pictus*), the melanophores in the 'Rhombus' of the 'Hirnhaut' taken from a particular place under the skull and removed with it, showed, when removed from the body and placed in salt solution, a remarkable response to the stimuli of

light and darkness. In the body the melanophores are contracted, when removed they expand. When they are now illuminated they show a complete contraction in from one to three seconds.

Melanophores have, however, also been found which when removed from the connections with the body failed to respond to the stimulus of light. Spaeth (13 b) could observe no case in which the contraction of the melanophores of isolated *Fundulus* scales could be unequivocally attributed to the action of visible light. Carlton ('03) found that when bits of the skin of *Anolis* were removed that the melanophores invariably contracted, and in the same length of time when they were placed in darkness as in light. In the normal animal light causes the pigment cells to expand. Biedermann ('92) asserts that when bits of skin are removed from *Rana temporaria* and *Hyla arborea* that the melanophores contract. Biedermann believes this to be due to the failure of the circulation of the blood. Carlton believes the contraction in *Anolis* to be due to the lack of nerve control.

A word further may be said here about what others have found concerning the action of ultra-violet light. Hertel ('07) found that, in *Triton taeniatus* and in young *Loligo*, contraction was brought about by the ultra-violet rays. If the illumination is not continued too long (10 to 15 minutes) this contraction was found to be reversible. Spaeth's results in this connection have already been given. Most recently Torracca ('13) has shown in another *Triton* (*Molge cristata*) that ultra-violet light brings about a contraction of the melanophores, both in the normal animal and in excised portions of the body and pieces of skin.

In all of these experiments the illumination with ultra-violet light resulted in a contraction of the melanophores. Spaeth does not state whether the melanophores of *Fundulus* respond normally to light and darkness, though he says the fish can be made to assume a pale condition by placing them over a white background, and a dark one by placing them over a black background. Torracca kept some of his animals for various lengths of time in darkness before he removed the skin for illumination

with the ultra-violet rays, and it is therefore to be assumed that darkness brings about an expansion of these melanophores, as in the Axolotl larvae (Babak) and in *Diemyctilus* (Rogers, '06). But as we have seen the melanophores of *Amblystoma* larvae expand in the light and yet the ultra-violet rays cause them to contract. When whole larvae, normal and eyeless, are illuminated with an arc-light the melanophores contract, if they are previously expanded, and are not affected, if they are previously contracted. This would seem to point to a specific effect of these rays, and to be in support of the view expressed by Torraca that perhaps this response of the melanophores is an adaptive one.

REACTIONS TO SOLUTIONS

Only brief mention will be made of a few experiments in this connection, since, in but one or two cases, the results were negative. After Spaeth ('13 b) had obtained such interesting results with the melanophores of the isolated scales of *Fundulus* by placing them in different ionic solutions, it seemed reasonable to suppose that the melanophores of *Amblystoma* larvae might also show reactions to immersion in such solutions. Accordingly, various solutions of sodium and potassium chloride were tried, particularly since Spaeth has found that these cations in combination with various anions produced expansion and contraction respectively. Various concentrations of these solutions were tried, viz, a normal solution, 0.5 N, 0.2 N, 0.1 N, 0.03 N, and 0.05 N solutions, also 0.65 per cent and 0.325 per cent NaCl and 0.8 per cent and 0.4 per cent KCl. The results of placing pieces of *Amblystoma* larva skin in these several solutions were all negative, the melanophores showing no response and remaining as they were when the skin was removed. Solutions of other substances were also experimented with, viz., chloretone (0.01 per cent and 0.02 per cent), atropin, (1 per cent), atropin sulphate (1 per cent to 4 per cent) and curare (0.1 per cent, 0.2 per cent and 1 per cent). Chloretone is the only one of these drugs that has any visible effect on the melanophores. When bits of skin are placed in a solution of chloretone the melanophores invariably show a $\frac{1}{2}$ to a $\frac{5}{8}$ expansion within thirty minutes or

so, although when first placed in the solution they are all completely contracted. In only one case out of five did the melanophores show a partial expansion in a 2 per cent solution of atropin sulphate, but, since none of the other experiments with this solution or with any of the others showed this effect, this case is believed to be accidental.

Experiments were also carried out in which the whole larvae were placed in the various solutions. Solutions of chloretone, atropin and curare were used. Larvae placed in 0.01 per cent and 0.02 per cent solutions of chloretone are narcotised, in the latter always, and in the former usually. As Harrison ('04) has shown, frog tadpoles may be kept in such solutions for several days with the complete cessation of all movement other than that of the heart. *Amblystoma* larvae can also be kept in these solutions of chloretone for several days, without any ill effects being apparent, larvae having been kept for as long as nine days in a 0.02 per cent solution, the rate of the heart beat being only very slightly retarded (Laurens '14 c). When the larvae are transferred to tap-water they completely recover from the narcotisation in a very short time.

The melanophores of larvae that have been placed in these solutions of chloretone very soon show a completely expanded condition. They begin to expand, whether the larvae are in the light or in the dark, in about twenty minutes, and in an hour are all completely expanded. When the larvae are transferred back to tap-water it is some time before the melanophores again contract. In the dark this contraction does not take place in less than twelve hours, and larvae placed on white backgrounds in bright diffuse light often do not show the characteristic state of the melanophores for two or three days.

The action of the chloretone on the melanophores, whether in the body or whether isolated, is to cause them to expand. The bearing of this on the part played by the nervous system in the reaction of the melanophores will be taken up later. A 1 per cent solution of atropin sulphate also brings about an expansion of the melanophores in all larvae placed in it, in light or in darkness, in about thirty minutes, although this solution has no effect

on the melanophores of isolated bits of skin. The larvae are not killed if they are not kept too long in the solution and the rate of the heart beat is not at all, or only slightly, retarded during a short stay. The melanophores will contract again when the larvae are transferred back to tap-water, and the larvae always appear not to have suffered any ill after-effects. The time the melanophores take to contract again after being placed in tap-water is about the same as in chloretone.

Hermann ('86) placed frog tadpoles in a solution of curare in attempts to paralyze them. He found that the larvae swam about for eight days in a tolerably strong solution. They behaved, he says, the same way in a solution of morphine. *Amblystoma* larvae have been placed in a 0.2 per cent and in a 0.1 per cent solution of curare. The first solution is just strong enough to render the larvae immotile. In neither solution do the melanophores remain contracted when the larvae, blinded and normal, are placed in darkness. That this is due to other causes than rendering the nerves powerless to conduct impulses to the melanophores owing to the curare affecting the nerve end connections is probable, as will be shown later under the discussion of the control of the central nervous system over the movements of the melanophores.

At this time experiments were in progress concerning the effects on the rate of the heart beat of placing larvae in different solutions of sodium and potassium chloride (see Laurens '14 c). It was therefore decided to note also the effects of these solutions on the melanophores of the living larvae. Various solutions, in addition to those enumerated above for the skin cultures, were used and also a Ringer solution. While nothing came out of these experiments as far as the melanophores are concerned it gave an opportunity to note some of the more obvious effects of these solutions on the larvae. Overton ('99, p. 115; see also Philip '13, p. 85) has stated that tadpoles of various European species of *Amphibia* (he mentions *Rana temporaria*, *Bufo variabilis*, and *Bombinator igneus*) will live indefinitely in a 0.6 per cent NaCl solution, which is presumably isotonic for these larvae. However true this may be for European tadpoles, it cer-

tainly is not so for the larvae of *Amblystoma*. Wilson ('97) has shown that in Ringer solutions and in 0.6 per cent NaCl, larvae of *Amblystoma punctatum*, *Rana temporaria* and *Chorophilus triseriatus* will not develop very far; and Jenkinson ('08) showed that the same is true if frog tadpoles in various isotonic solutions. When *Amblystoma* larvae, after having attained a certain stage of development, either just before or after they have begun to feed, are placed in various solutions they also will not live for any very great length of time. The following solutions have been tried: 0.02 N, 0.01 N, 0.05 N, 0.2 per cent, 0.3 per cent, 0.325 per cent, 0.4 per cent, 0.5 per cent, 0.6 per cent, and 0.7 per cent NaCl, the same normal solutions of KCl and 0.4 per cent, 0.6 per cent and 0.8 per cent. In all of these the larvae will live but a relatively short time, longer in the weaker solutions than in the stronger, and the older larvae longer than the younger. All of the solutions caused a spasmodic twitching of the body and if the larvae are caused to swim rapidly by mechanical stimulation the swimming movements always end in a complete tetanus of the whole body. The large larvae of *punctatum* and *opacum* (35 to 55 mm. long) show a much greater resistance than do the smaller (18 to 25 mm.) larvae of *punctatum*. One *opacum* larvae (40 mm. long) was kept for fourteen days in a 0.65 per cent solution of sodium chloride. For nine days the larva appeared normal, eating with avidity small Crustacea and smaller *Amblystoma* larvae and frog tadpoles, and giving only now and then a slight indication of spasmodic twitching and incomplete tetanus. After nine days the body cavity was observed to be swollen and in five more days the larva was dead. The KCl solutions were always more quickly toxic than the NaCl.

Backman ('12), Backman and Runnström ('09 and '12) and Backman and Sundberg ('12) have shown that the young larvae of *Rana temporaria*, *Bufo vulgaris* and *Triton cristatus* have an osmotic pressure which is much lower than that of the adults, and which increases with the growth of the larvae. The osmotic pressure just after fertilization they found to be as low as one-tenth that of the adults, and only after the thirty-fifth day of development to be equal to that of the adults. There can be

no reasonable doubt that this general fact holds also for the larvae of *Amblystoma*. It was for this reason that the large number of different solutions mentioned above were used in the attempt to find one which would be isotonic, or approximately so, with the tissues of the larvae, and in which they might therefore live.

As was mentioned above these experiments as far as the melanophores were concerned were negative. For about twenty-four hours after being placed in any of the solutions the larvae showed all of the responses to light, darkness and background as when in tap-water. After this, in the case of the weaker solutions a little later, the larvae lose this ability, and the melanophores are found in all stages from complete expansion to complete contraction, though in the majority of cases they are expanded. The sodium and potassium ions showed absolutely no specificity in causing expansion and contraction.

In a Ringer solution (Clark) the older larvae will live for as long as twenty days without showing until a few days before they die any ill effects. Furthermore, all the changes of the melanophores take place as though the animals were in tap-water. Suddenly, however, they appear sickly, although they have eaten regularly and if not soon transferred to tap-water, they will die. Sometimes, in larvae kept in this Ringer solution the symptoms of spasmodic twitching of the fore limbs and of the head make their appearance, particularly if the larvae are stimulated to swim rapidly and vigorously for some time. Often these swimming movements are followed by a complete tetanus.

There seems then to be no solution (other than very dilute ones of NaCl) in which *Amblystoma* larvae will live as they will in water, even though this is certainly not isotonic with their tissues. As Backman and his co-workers have pointed out, there must be some regulatory apparatus by means of which the ability of amphibian larvae to live in water not isotonic with them is made possible, since diffusion through the skin takes place, this apparatus being in all probability the urinary system.

REACTIONS TO TEMPERATURE

Changes in temperature have been shown to have an effect on the melanophores of various animals. In general, low temperatures cause an expansion of the pigment cells and high temperatures a contraction. Knauanthe ('91) observed that *Rana temporaria* and *R. esculenta* and *Bufo variabilis* turn dark when kept at low temperatures. Biedermann ('92) stated that warming frogs causes the black pigment cells to contract, while cooling them causes them to expand. Ehrmann ('92) found that when a metal capsule filled with warm water is touched to the skin of a frog that the melanophores contract, Fischel ('96) that the larvae of *Salamandra maculata* in cold water (6 to 7°C.) are dark, while in warmer water (15 to 18°C.) they are pale. Parker and Starratt ('04) noted that in *Anolis carolinensis* a high temperature (40 to 45°C.), produces a contraction of the pigment cells which is irrespective of illumination, and a low temperature (10°C.) an expansion, and later Parker ('06) found that a high temperature (32°C.) causes a proximal migration of the pigment, while a low one (15°C.) causes a distal migration in the lizard *Phrynosoma blainvillei*. Rogers ('06) also found that a low temperature (10°C) brings about a dark coloration of *Diemytilus viridescens*, while a high temperature (35 to 40°C.) has a lightening effect. Hargitt ('12), however, described rather interesting and slightly different results from those of other investigators. He noted that a high temperature brings about a lightening of the coloration of the frogs *Hyla versicolor* and *H. arborea*, but could observe no definite effect of a low temperature. Finally Spaeth ('13 b) showed that high temperatures (30°C.) produce contraction of the melanophores in the scales of *Fundulus*.

In solitary disagreement with these general results are those of von Frisch ('11 a and '11 b). In *Phoxinus laevis*, a high temperature (35°C.) brings about, instead of a contraction, an expansion of the melanophores. At 15°C. the skin takes on a medium coloration, and at 3 to 5°C. the melanophores are all completely contracted. These effects von Frisch observed by warming one side of the body and cooling the other. Later von

Frisch ('12) found that the colored pigment cells—red and yellow—of *Crenilabrus pavo* and *Trigla corax* also contract when the temperature is lowered and expand at higher temperatures. These temperature effects von Frisch regards as purely local and in no way connected with the nervous system. In dead fish an opposite effect of high and low temperatures was found to exist. Here the warmed side of the fish (35°C.) is paler than the cold side (15°C.). This contraction brought about by high temperatures is referred by von Frisch to the lack of oxygen, but Spaeth ('13 b) has easily refuted this by showing that at 29°C. *Fundulus* scales placed in water where there is an excess of oxygen always contract.

Most of the experiments dealing with temperature have been carried out in connection with light and darkness, and it is interesting to look briefly at the results which have been obtained. Knauanthe ('91) noted that when frogs were frozen they never afterwards took on a light coloration, even though they were thawed in the sun. Biedermann ('92) who places very little importance on the effect of light in bringing about movements of the pigment thinks that temperature plays a very important rôle. Parker and Starratt ('04) found that at a low temperature (10°C.) the resultant expansion of the melanophores cannot be influenced by darkness and concluded that at this point temperature is the controlling factor. At high temperatures (40 to 45°C.) the resultant contraction of the melanophores can also not be influenced by light and therefore again at this point temperature is the controlling factor. But at intermediate temperatures the expanding effect of light and the contracting effect of darkness make their effects noticeable, although the effects of temperature are still apparent in their influence on the rate of the color changes. Parker ('06) found also that in *Phrynosoma blainvillei* at 19°C. in the light the melanophores expanded and in darkness contracted. Light, therefore, and low temperatures produce expansion, and darkness and high temperatures contraction, but since when a low temperature (15°C.) is combined with darkness contraction follows, and when a high temperature (32°C.) is combined with light expansion of the melanophores

takes place, Parker concludes that light, or its absence, is a more effective stimulus of the melanophores than heat or cold.

Rogers' ('06) results are somewhat different from those of others owing to the fact that in *Diemytilus* light brings about a contraction of the melanophores (as in frogs) and therefore low temperature and darkness bring about expansion; high temperature and light contraction. The effects of illumination Rogers found to be inhibited by temperature, and he has shown that when combinations of a high temperature (35 to 40°C.) are made with darkness—these having opposite effects—neither the contracting effect of the high temperature nor the expanding effect of darkness outweighs the other, but that an "ordinary" coloration is the result. The same thing is obtained when a low temperature (below 30°C.) is combined with bright light.

Hargitt's results in this regard are also slightly different from others. As noted above he could observe no definite effect of low temperatures on the coloration of tree frogs. As to illumination he finds that light brings about a contraction of the melanophores (just as in all frogs) though he quotes exceptions to this general result. But darkness he found to have no appreciable effect on the pigment cells, which is altogether against the views of others. But it must be remembered that Biedermann was always strongly against ascribing to light any very important share in bringing about changes in the coloration of frogs. He observed these changes but thought that they were subordinate to other conditions such as temperature, tactile stimuli, etc. Hargitt's results are in agreement with this view of Biedermann's. But, on the other hand, although Hargitt finds that a high temperature brings about a lightening of the coloration of tree frogs, a low temperature has no definite effect. Biedermann, however, regards temperature, both high and low, as one of the most important conditions in bringing about changes in the pigment cells of frogs. Since Hargitt finds that neither darkness nor low temperature, both of which would be expected to bring about an expansion of the melanophores, has any visible effect, a comparison of low temperature combined with light, and of high temperature with darkness would have been interesting in order to

see, whether, although without direct effects, low temperature and darkness could not be shown to inhibit respectively the effects of light and high temperature.

Amblystoma larvae show responses of the melanophores to differences in temperature and these can be shown to be related to illumination. When the larvae are placed in a cold room at various low temperatures from 4 to 12°C. the melanophores always completely expand, whether the animals be kept in total darkness, or brightly illuminated on indifferent or on white backgrounds. Above 12°C. or thereabouts, and certainly above 15°C. the primary contractions of the melanophores in darkness and in the light on white backgrounds, and the 'secondary' responses of contraction in light on indifferent backgrounds come about as at ordinary room temperatures (19 to 23°C.). The effects of high temperature are not so easily demonstrable as those of low. Up to 38°C. no effect can be seen, but above this, by gradual warming, a contraction of the melanophores can always be brought about, though not a complete one, the melanophores never contracting beyond a condition of $\frac{1}{4}$ expansion and usually not beyond $\frac{1}{2}$.

With respect to the effects of low and high temperatures in connection with light and darkness, it was found that up to 12°C. temperature is more efficient than darkness, the melanophores always becoming and remaining expanded in the dark as well as in the light. Above that and up to 15°C. it is questionable, but at and above the latter temperature darkness always showed itself the more potent stimulus and produced a contraction of the melanophores, though after a longer time than at ordinary room temperatures. In the light too at this temperature (15°C.) expansion came about more quickly than at ordinary temperatures. An inhibiting effect of high temperature can be more easily shown than an actual direct contracting effect. In water above 32 to 35°C. the melanophores show a slightly quicker contraction time when placed in darkness than they do in water of ordinary temperatures, and these temperatures retard appreciably the expansion of melanophores in larvae that are transferred from darkness to light.

When pieces of skin are removed from the body and mounted in drops of various fluids as mentioned above they lose all ability to respond to the stimuli of light and darkness, with the exception of the short invisible rays. One might expect that the same would hold true for temperature. But such pieces of skin in which the melanophores when removed are expanded show after a few minutes when heated on a warm stage to 38°C. or higher, a decided, though never complete, contraction. In the same way bits of skin in which the melanophores are at first contracted show at 10°C. and lower a decided, though also never complete, expansion. These contractions and expansions of the melanophores of isolated pieces of skin are reversible to a slight extent. When pieces of skin are placed in chloretone (0.01 per cent and 0.02 per cent) the contractions of the melanophores when the skin is warmed will not take place even when the temperature is as high as 45°C.

REACTIONS TO ELECTRIC CURRENTS

It has frequently been shown that chromatophores respond to electrical stimulation, both direct and indirect. Spaeth ('13 b) has recently given a list of some of the papers dealing with these responses of the melanophores of various animals to electrical stimulation and there is hardly any need to call attention here to but a few of them, and to briefly give the results of those that seem to be most important. Later under the discussion of the part played by the central nervous system in bringing about changes in the form of the pigment cells this matter will be referred to again.

Brücke ('51, '52), in his classic work on the color changes of the chameleon, found that when the cut spinal cord, or a mixed nerve, is strongly stimulated by an electric current the melanophores contract (see Van Rynberk '06, p. 447). Bert ('75) pointed out that not only would the stimulation of a nerve bring about the contraction of the melanophores but that also the stimulation of an excised piece of skin produced the same effect. Biedermann ('92) observed that stimulation of the cut sciatic

nerve with an interrupted faradic current of sufficient strength and duration brought about a paling of the skin. Keller ('95) corroborated Brücke's and Bert's results on the stimulation of the cut nerves of the chameleon. He found that strong currents continued for a long period of time were necessary, but that in about $1\frac{1}{2}$ minutes after the current is broken the portion of the skin innervated by the cut nerve is again dark. He also demonstrated that stimulating the posterior portion of the cut spinal cord has the same effect as stimulating the nerves.

Von Frisch ('11 a and '12) has shown that electrical stimulation, both of the cut central nervous system and of the melanophores directly brings about a contraction of the colored and black pigment cells of fishes. Spaeth has shown that the same is true of the melanophores of *Fundulus*. Winkler ('10) noted that the stimulation of a bit of excised frog skin (*Hyla*) with an induced current will bring about a contraction of the melanophores; but that stimulation with a constant current results in an expansion of the melanophores, which is followed by a contraction as soon as the stimulation is ended.

When large larvae of *Amblystoma opacum* (35 to 55 mm. long) with expanded melanophores are placed in a watch glass and stimulated with an interrupted current (Harvard inductorium, with 1 or 2 'red seal' dry cells) a contraction of the melanophores can be brought about after a long-continued stimulation and with a sufficiently strong current. The current must be allowed to act for at least fifteen minutes. When various portions of the body are cut out and directly stimulated, either with the central nervous system intact or destroyed, a slight contraction of the melanophores is usually induced.

When small pieces of skin are removed from the head region or dorsal portion of the body and placed in small drops of sodium chloride or of tap-water and stimulated with a faradic current the melanophores contract, though here again the current must be strong and long continued.

An induced current then, when of sufficient strength and duration causes the melanophores to contract. This contraction is only to a slight extent reversible and only when the skin is

stimulated with a constant current. When the melanophores are simply left alone after stimulation with the induced current no expansion can be detected.

When whole larvae and small pieces of skin are stimulated with a constant current, instead of the melanophores contracting, they expand maximally. The current does not have to be very strong, not more than 3 M. A. being necessary. The larvae were placed in a wooden trough, 40 cm. long and 7 cm. deep, filled with water and at each end of which was placed a platinum electrode. When a constant current of 1, 2, or 3 M. A. is passed for a few seconds to a minute in either direction through a larva in which all the melanophores are completely contracted,—the whole procedure being carried out in darkness—in from ten to twenty-five minutes afterwards the melanophores are $\frac{1}{4}$ expanded, and in thirty-five to forty minutes completely expanded. If the animal is left in the dark, or placed in diffuse light on a white background, in ten to twenty hours the melanophores are again contracted.

When bits of isolated skin with contracted melanophores are thus stimulated on a glass slide with a current of from 3 to 4 M. A., and for about five minutes, the melanophores all expand. All attempts to demonstrate a greater effect of one pole in these expansion effects resulted negatively. No matter in which direction the current flows through the body or through the skin, all the melanophores expand. In two cases out of ten experiments with bits of isolated skin it was thought that the expansion at the cathode began earlier and was more extensive than at the anode, but this is not sufficient evidence for ascribing to that pole a greater effect.

THE INFLUENCE OF THE NERVOUS SYSTEM ON THE REACTIONS OF THE MELANOPHORES

A discussion of the part played by the nervous system in the reactions of the melanophores to various stimuli must be given consideration in any paper dealing with the physiology of the pigment cells. Babak has described in the Axolotl the effects of direct stimulation with light—no attempt being made to distinguish between a stimulus affecting the melanophores

themselves, and one acting on nerve endings in the skin and secondarily, after passing through a reflex arc, affecting the melanophores—to be exactly opposite to those brought about by indirect stimulation through the eyes.

Various experiments, which will now be briefly mentioned, were carried out to see how relatively important are the two methods of stimulation: direct—the stimulus acting upon the melanophores themselves; and indirect—the stimulus acting upon nervous end organs, the eyes or sensory endings in the skin. Since in the whole animal—seeing and eyeless—with intact central nervous system it is impossible to say whether the stimulus producing the ‘primary’ responses acts at all on supposed sensory endings in the skin, thereby setting up impulses which are conducted to the melanophores along a nervous pathway to the central nervous system and out again to the pigment cell, or whether the pigment cell is directly stimulated, special experiments are necessary to throw light on this matter. Of course, the experiments which have been so far described for isolated pieces of skin have shown that the melanophores may be directly stimulated when their connection with the body is broken. But it is interesting to endeavor to find evidence as to whether, when intact in the body, with normal blood connections, etc., but with the nervous connections severed, the responses of the melanophores will still come about and be of the same nature as when the nervous connections are normal.

The experiments carried out with this view may be most conveniently considered in the same grouping as those which have already been described.

1. Experiments with light

It has been shown at considerable length above how the melanophores of the larvae of *A. punctatum* and *opacum*, seeing and eyeless, respond primarily to light by expanding and to darkness by contracting. Also how in a few days the expanded condition of the melanophores of normal larvae in the light gives way to a tonic condition of contraction, and how the contracted

condition of the melanophores in darkness gives way to a lasting expanded condition. Furthermore, it has been shown that these 'secondary' responses of the melanophores do not take place in the eyeless or in the blindfolded larvae. Also, that normal seeing larvae respond to differences in background in so far as on a white background in bright diffuse daylight the melanophores contract, and on a black one expand, or remain expanded.

These results lead indisputably to the conclusion that the 'secondary' responses of the melanophores of normal seeing larvae are due to nervous activities. Also that these nervous activities are set up by stimuli received by the retinae and transmitted as impulses to the central nervous system where they are transformed and sent out along motor nerves to the pigment cells.

At this point the question can be asked as to whether any portion or portions of the central nervous system can be shown to have a controlling influence over, or to be a 'center' for, these 'secondary' changes of the melanophores, and also in general for the responses of the pigment cells to light. Biedermann ('92), repeating experiments of Steiner's, has shown that in *Hyla* the optic thalamus is to be regarded as the center of innervation of the black pigment cells. In the brown grass frog (p. 503) there seems to be in addition a second subordinate center in the deeper portions of the brain, perhaps even in the spinal cord. Von Frisch ('11 a and '12) has demonstrated that in the anterior portion of the medulla oblongata there is a center, the stimulus of which causes a contraction, its destruction, the expansion of the melanophores of *Phoxinus laevis* and of the colored pigment cells of *Crenilabrus pavo* and *Trigla corax*. In addition to this chief center von Frisch showed that there is a second, subordinate center in the spinal cord, from which, after the death of the animal, impulses go out causing a contraction of the melanophores (also in *Salmo fario*). The destruction of this subordinate center results in the expansion of the pigment cells. Spaeth ('13 b, p. 549) also mentions briefly the fact that, in *Fundulus*, some hours after they have been decapitated, in consequence of which all the melanophores are expanded, the melanophores are

all contracted again. As Biedermann (p. 478) remarks, this contraction of the black pigment cells after death seems to be a wide spread phenomenon, but it is one which he ascribes to the failure of the circulation of the blood and not to nervous influences. It may be mentioned that this contraction of the melanophores after the death of the animal cannot be observed in *Amblystoma* larvae. If larvae are decapitated, or the body cut completely through at various levels, the melanophores neither expand or contract, but remain in the condition in which they were in the living animal. Also larvae that have died show the condition of the pigment cells characteristic of the condition of illumination, etc., under which they were. In short, the melanophores show no spontaneous post mortem changes.

But when the spinal cord of larvae whose melanophores are contracted is cut through at various levels, from just in front of the brachial plexus and posteriorly, there is invariably to be observed, after a few minutes, a complete expansion of all melanophores even though the animal be kept in the dark, just enough illumination being used at the time of the operation to perform it, and which does not under ordinary circumstances have any visible effect on the pigment cells. If the larvae now are kept in darkness the melanophores soon (4 to 8 hours) contract again. When such larvae are placed in bright diffuse daylight the melanophores all over the body, both anterior and posterior to the cut expand in the same time as in normal seeing larvae (2 to 3 hours). Also when these larvae are allowed to remain in light or in darkness the 'secondary' responses come about just as in normal seeing larvae. In addition, when the larvae are placed on white backgrounds the melanophores also still contract. It is well known, of course, that the melanophores are under the control of the sympathetic system as well as of the spinal system (Carlton '03, Hooker '12) and the results here detailed simply show that this is true of the pigment cells of *Amblystoma* also. Since after the spinal cord has been cut through the responses to background still take place it is evident that, in order that the melanophores posterior to the cut may be brought to contract when the larvae are placed on a white back-

ground and when allowed to remain in the light on an indifferent background, and to expand secondarily when allowed to remain sometime in darkness, the nervous pathways (sympathetic) must leave the spinal cord by means of the spinal nerves and go over into the sympathetic chain, from where running anteriorly and posteriorly they finally leave it to innervate the pigment cells.

Experiments were carried out on large larvae to locate where these sympathetic fibers leave the spinal cord to pass out by means of the spinal nerves to the periphery. The method employed was to make successive sections of the spinal cord, each successive section being further anterior than the last, several sections thus being made in the same individual; or to make single sections at different levels in various animals. In most of these experiments chloretone was used to narcotise the larvae, though in some which have already been mentioned this was not done, since chloretone always causes the melanophores to expand, and it was desired to see what the effects of transecting the cord themselves were. Without chloretone narcosis transecting the cord at any level produces a temporary expansion of all melanophores, this expansion giving way under proper conditions (darkness) to contraction. Even when the successive sections are extended up into the brain, the various portions being in turn cut through until the prosencephalon itself is divided, the subsequent contraction after the expansion caused by the transection still takes place. The conclusion may be drawn therefore that in *Amblystoma* larvae there seems to be no center for the contraction of the melanophores, in the sense that Biedermann describes as existing in the frog.

When the cut is made through the central nervous system anterior to the first or second vertebra the response to a white background ceases, as does also the 'secondary' expansion in darkness and the 'secondary' contraction in diffuse light on an indifferent background. These results show that the responses of the melanophores to backgrounds, and the secondary responses under constant conditions of illumination or darkness are essentially nervous, and in addition, that they are under the control of the sympathetic system and that the fibers of the sympathetic

leave the central nervous system at the level of the first or second spinal nerves.

There is additional evidence to be obtained from these operative experiments upon the central nervous system. When the spinal cord, in addition to being cut through, is bored out posteriorly, and when the cut is posterior to the exit of the sympathetic fibers which control the pigment cell changes, the primary and secondary responses to light and darkness take place over the whole body just as in normal seeing larvae, as well as the contraction of all the melanophores when the larvae are placed in bright diffuse light on a white background. When the portion of the spinal cord anterior to the cut, or when the entire spinal cord is bored out, the secondary responses of course no longer take place, nor do the contractions of the melanophores on a white background. The primary responses to light and darkness are, however, perfectly normal, taking place in the same average length of time as in eyeless larvae, and the melanophores remain in the characteristic conditions for light and darkness no matter how long they are kept there.

These larvae with the central nervous system destroyed have been kept for ten days, after which they were preserved. They remain perfectly still unless mechanically stimulated, though the rate of the heartbeat is not measurably decreased and the blood can be observed to be flowing as usual through the gills, in the skin, and in the vena abdominalis back to the heart.

2. Experiments with solutions

In many of the papers on the physiology of the chromatophores the effects of curare on the pigment motor nerves have been considered. It has been shown that curare does not affect or influence the color changes in any measurable degree. Biedermann ('92) found that when a frog is weakly poisoned with curare, and the cut sciatic nerve tetanized, a distinct paling of the skin of the leg takes place, and that in fifteen to thirty minutes the melanophores are completely contracted. A strong dose of curare is a poison and causes the melanophores to expand, but only temporarily. Biedermann also found that when curare

came in contact with the skin it had a direct contracting effect, though as was pointed out above curare has no effect on the melanophores of bits of *Amblystoma* larva skin immersed in it. Bert ('75) earlier found that curare does not affect the pigment motor nerves of the chameleon, and that the stimulation of the nerves brings about a contraction of the melanophores just as in noncurarized animals. Steinach ('91) also observed that curare does not affect the responses of the melanophores of frogs to light.

Hermann's ('86) experiments on frog tadpoles have already been referred to (p. 602). *Amblystoma* larvae placed in a 0.2 per cent solution of curare do not swim about, but remain perfectly still, except when vigorously stimulated mechanically, where they will respond with a weak movement of the tail. Under such conditions the melanophores do not contract, when the larvae are placed in darkness, even when kept there for a week. In these larvae curare does not have a direct contracting effect on the pigment cells either when they are isolated or when they are in the body, and the intact cells lose the ability to contract when they are placed in darkness. This failure on the part of the pigment cells to contract may be due to the direct effect of the solution on the animal. The curare solution probably brings about the asphyxiation of the larvae (though it takes more than a week for a 0.2 per cent solution to prove fatal) and the consequent increase in the amount of CO_2 in the blood may cause the melanophores to remain expanded. Biedermann has shown that in frogs asphyxiation (increased CO_2) brings about an expansion of the melanophores, while a decrease in oxygen causes them to contract.

If a small amount of a 1 per cent solution of curare is injected into a larva it becomes in a few minutes absolutely motionless. If placed in darkness the melanophores contract and when transferred to the light they expand. This experiment offers no proof that the curare does not affect the nerves, since it has already been shown that the melanophores will continue to contract and expand after all nervous connections with them have been destroyed.

Other nerve poisons were also experimented with. When a drop or two of a 1 per cent solution of strychninum nitricum is injected into a larva the characteristic strychnine cramp takes place after a short interval. A few minutes after this cramp begins and for as long as it lasts the melanophores are contracted. When the strychnine dose is light the larvae recover quickly from its effects and the melanophores then react normally again to light and darkness. The result of this simple experiment points incontrovertibly to the conclusion that the spinal nerves also have some control over the melanophores, since we know, from the work of Verworn and his co-workers, that the seat of strychnine poisoning is in the dorsal horn cells. That the spinal connections, however, are not relatively important has been shown by the experiments in which all the responses to light, background and darkness take place when these connections were destroyed.

Experiments were also made with nicotine, a specific sympathetic nerve poison. Carlton ('03) in his work on the color changes of *Anolis* made use of this drug. He found that when it was injected into the body cavity, it produced a contraction of the pigment cells, by removing their nervous control. When a few drops of a 0.01 per cent solution are injected into the body cavity of *Amblystoma* larvae both into normal larvae, and into those in which the central nervous system has been destroyed, the melanophores do not contract, but continue to react to light and darkness as usual. It is of course not absolutely certain that the nicotine in this case affects the sympathetic system, though it is highly probable. If it does it is further evidence of the much greater importance of direct, as compared with indirect, stimulation of the pigment cells.

3. Experiments with temperature

The question must arise as to whether the effects of temperature are to be regarded as being due to a strictly local stimulation of the pigment cells or to an indirect one taking place by the stimulation of the nerve endings in the skin. Biedermann (pp. 477 and 487, see also p. 508), who regarded temperature as one of the most important conditions to be taken into account in

the consideration of the responses of the melanophores of *Rana temporaria* and *esculenta*, was of the opinion that it acted directly on the melanophores and not indirectly through the central nervous system. An indirect reason for this he saw in the fact that, when the principal nerve of an extremity is cut through, the soft parts removed, and the nerves running along the blood vessels also removed, the post-mortem contractions of the melanophores, which are due to the failure of the circulation, (p. 478) come about more quickly when the temperature is raised than happens at ordinary temperatures.

It has been shown above, by placing isolated pieces of skin from *Amblystoma* larvae in hanging drops and warming and cooling them, that temperature does affect the melanophores directly. But again it is interesting to see what the conditions in the body are when the central nervous system is destroyed. When this is done, the blood circulation being intact and as normal as possible under the circumstances, when the temperature of the water is gradually raised above 38°C. the contractions of the melanophores take place as usual. When cooled below 10°C. expansion always takes place. It has earlier been noted that when the bits of skin are placed in 0.02 per cent chloretone the contraction by warming cannot be brought about. The same thing is observed when the central nervous system is destroyed, the melanophores of such larvae remaining always expanded. This is additional evidence that chloretone exerts its expanding (anesthetic) effect not merely by means of the nervous system but also directly on the peripheral organs.

4. Experiments with electric currents

When the central nervous system is cut through and the cut surface stimulated with an induced current of sufficient intensity and duration a contraction of the melanophores results. It is of course not possible to say whether it is the stimulation of the spinal nerves or of the sympathetic nerve fibers that causes this contraction. It can also be brought about when the cut through the central nervous system is posterior to the exit of the sympa-

thetic fibers, which points again to the conclusion that, although the control of the spinal nerves over the melanophores is normally weak, they do have some control. In addition, as has already been pointed out, direct stimulation of the melanophores in the body and in isolated pieces of skin, also causes contraction.

The main effects of stimulation with the constant current have been noted. It produces an expansion of the melanophores both in the whole animal and in isolated pieces of skin. Now when larvae in which the spinal cord has been cut through are placed in a wooden trough and the current passed through them, the effect is the same as in the normal animal, the expansion of the melanophores taking place over the whole body. Again under these conditions no evidence of a greater effect of the positive or negative ions can be demonstrated. When a larva, in which the anterior portion of the nervous system has been bored out, is stimulated with the constant current, expansion of the melanophores takes place over the entire body no matter in which direction the current is flowing and the same is true when a larva in which the portion posterior to the cut, or the entire nervous system has been destroyed, is stimulated. From this there can be no doubt that the constant current acts directly on the melanophores. In addition it probably also acts indirectly, by means of impulses sent along the spinal and sympathetic nerve fibers.

DISCUSSION AND CONCLUSIONS

Normal seeing, and eyeless larvae of *Amblystoma punctatum* and *opacum* show exactly opposite conditions of the melanophores under identical conditions of light and darkness. This is a condition, however, which though permanent comes about secondarily. The primary responses of the melanophores of seeing and blinded larvae are identical. Darkness produces a contraction and illumination an expansion. According to Babak, in the larvae of *A. mexicanum* Cope (*A. tigrinum* Laurenti) the differences in the responses of the melanophores between seeing and blinded larvae are primary and always exist. In the Axolotl

the melanophores of the blinded larvae contract in darkness and expand in light, while the melanophores of normal seeing larvae expand in darkness and contract in the light.

A number of investigators have noted that blinding animals causes the melanophores to expand, but few have carried the matter further to see if in these blinded individuals the effects on the melanophores of illumination and darkness were still to be observed. To mention but a few instances, Rogers ('06) sectioned the optic nerves of *Diemytilus viridescens* and noted that the animals became permanently dark. Darkness normally produces in this animal an expansion of the chromatophores, as in the frog, but it would have been interesting to know how the melanophores of these blinded individuals behaved in darkness. Franz ('10) experimented with young flat-fish (*Pleuronectes platessa*). He records for these fish a highly developed response to differences in the nature and color of the background, but does not mention their reactions to light and darkness. When the young fish are blinded a maximal expansion of the chromatophores takes place. Šečerov ('10) showed that normal seeing *Nemachilus barbatula* exhibit a marked adaptability to background (color, tone, etc.). When placed in the dark they assume a dark red-brown color, and when placed in the light and illuminated from above they turn pale. When blinded fish are placed in darkness they assume a red-brown color (lighter than the normals), but when brought into the light they are dark red-brown, as blinded animals are, which have always been kept in the light. From this it seems that in the blinded animals there is a slight contraction of the chromatophores, at least of some of them, in darkness, and that light causes them to expand again. Eycleshymer ('14) has recently noted that the distribution of the pigment of decapitated *Necturus* larvae is the same as that in the normal larvae, but that the chromatophores are greatly contracted. Von Frisch ('11 a) found that all operations which render the eyes functionless (extirpation, cutting of the optic nerves, cutting the chiasmata, destroying the retinae) cause, in certain of the Cyprinoid fishes, (*Carassius vulgaris*, *Phoxinus laevis*) and in *Salmo fario*, a darkening of the whole body.

This darkening comes about in a few minutes and lasts for several weeks, after which the fish gradually assume their normal color. The blinded fish lose the ability to adapt themselves to background, but not the capability of spontaneous color change. In *Salmo* there is simply a darkening produced, the adaptability to bright backgrounds being lost. In these fish the light which is received through the eyes is the most important of the factors which have to do with the tonic contractions of the pigment cells, and when this is removed the pigment cells relax. The trout is not only dark when the eyes are removed, but also when normal animals are placed in darkness. In *Phoxinus laevis* (v. Frisch, '11 a and '11 c) the conditions are similar, though in the blinded fish the melanophores are not directly stimulated the changes in the pigment cells being brought about by the stimulation of certain sense cells in the epithelial covering of the 'Zwischenhirnventrikel.' In this fish there is a difference in the reactions of the chromatophores of the seeing and blinded individuals. The former respond to the brightness of the background, turning paler the brighter it is; but the blinded fish turn darker the greater the intensity of the light, and of course show no responses to differences in background. When the blinded fish are brought into darkness they turn pale. Illumination therefore has a directly opposite effect on the melanophores of the blinded fish to what it has on the pigment cells of the normal seeing individuals. Von Frisch further found that the extirpation of one eye in Cyprinoid fishes has a short temporary darkening effect over the whole body, and in *Salmo* it causes the opposite side to become permanently darker. The covering over of the eye with a black blind has in the trout the same effect as the extirpation of the eye. In *Crenilabrus pavo*, however, (v. Frisch '12) the conditions of the chromatophores in seeing and blinded individuals are not opposite, but identical; illumination causes expansion, and darkness a contraction, and in blinded fish more clearly than in the normal seeing ones.

Babak's views on the differences found between the melanophores of the seeing and blinded larvae of the Axolotl have several times already been referred to. But for the sake of

clearness and comparison the following quotation will be given here (Babak '10 a, p. 104).

Es lässt sich kaum darüber zweifeln, dass die Chromatophoren der Amblystomalarven in beiden Phasen ihrer Bewegungen—sowohl bei ihrer Ausbreitung als auch bei ihrer Zusammenballung—durch das Zentralnervensystem beherrscht werden, und zwar wird diese mächtige doppelte Innervation durch die Netzhäute bedingt. Den Netzhäuten muss man zweierlei entgegengesetzte Beeinflussung des Zentralnervensystems zusprechen, je nachdem dieselben beleuchtet oder verdunkelt werden. Die verdunkelten Netzhäute wirken ebenfalls positiv, d. h. bewegungsauslösend auf die Chromatophoren ein, wie die durch das Licht gereizten Netzhäute, aber im entgegengerichteten Sinne. Die Vernichtung der Netzhäute hat ganz verschiedene Folgen als ihre Verdunkelung, oder anders gesagt, die Netzhäute sind auch bei volligem Lichtabschluss tätig, und zwar in entgegengesetzter Richtung, als bei starker Beleuchtung.

And later, on p. 105:

Man wird hier wohl nicht zweierlei nervöse Hemmungswirkungen, einander entgegengesetzt, annehmen, sondern zwei tonische Innervationsarten der beiden Bewegungsphasen der Chromatophoren; die Chromatophoren-Ausbreitungsinervation entspringt den verdunkelten Netzhäuten und ist zuweilen so stark, dass die Tendenz der gleichfalls verdunkelten Chromatophoren sich extrem zusammenzuballen überwindet und Verdunkelung der Haut hervorruft; die Chromatophoren-Zusammenballungsinervation entspringt den beleuchteten Netzhäuten und ist zuweilen so stark, dass sie die Tendenz der gleichfalls beleuchteten Chromatophoren sich extrem auszubreiten überwindet und Erbleichung der Haut bewirkt.

That this explanation will not apply to the conditions which exist in the larvae of *A. punctatum* and *A. opacum* must be clear from the results given above. Babak (pp. 97 and 99) mentions only the differences between the state of the pigment cells of normal and blinded Axolotls, and it is therefore to be inferred that these differences do not come about secondarily but that they represent the primary and only response of the melanophores. Hooker ('14 a), however, mentions a secondary expansion of the melanophores of tadpoles that have been kept for more than six hours in darkness. As we have seen, in *A. punctatum* and *A. opacum*, it is only after a few days of constant illumination or darkness that the opposite conditions in the see-

ing larvae from those previously found there, and always found in the eyeless larvae, take place. The secondary contraction of the melanophores of the seeing larvae which takes place after a few days of constant illumination (interrupted at night) is only a partial one. It might be assumed that the constant illumination of the retinae causes impulses to be set up, the end effects of which are opposite to those of direct stimulation, and that under constant conduction of these impulses to the pigment cells a contraction is brought about. The fact that the secondary contraction is not a complete one points to the view that there may be a conflict, as it were, going on between the two kinds of impulses, and that that one set up by the indirect stimulation finally gains the upper hand and the pigment cells are brought to contraction. It must be admitted (see Table 2) that it is the general function of these pigment cells to expand when illuminated, both when indirectly stimulated as well as when directly, since seeing larvae show the primary expanded condition of the melanophores when illuminated more quickly than the eyeless larvae do. Later, perhaps owing to the photochemical action on the retinae of continued illumination, impulses are set up which have an opposite effect and the pigment cells are brought to contract.

The question as to which condition of the melanophore is to be regarded as the active and which the passive has a certain significance. It seems most plausible, speaking generally, to regard the expanded condition of the pigment cells as the relaxed (passive) one, and it might be said that for a melanophore to contract and to remain contracted a nervous impulse set up by the light stimulus has to work against two forces, the one the result of the direct stimulation of the pigment cell which causes it to expand, and the other the general tendency of the pigment cell to relax, or in other words to expand. But in darkness the primary response of the melanophore is also to contract. Certainly from this, one might advance a strong argument for the view that the contracted condition of the melanophore is the passive one, or the one assumed when the pigment cell is at rest, and that the protoplasm of the pigment cell by simply relaxing

becomes balled up into a single point, just as an *Amoeba* which is rounded is more at rest than one in which the protoplasm is pushed out into pseudopodia, (leaving out of consideration the means by which, or the manner in which, the pseudopodia are caused to protrude). But none of this explains the 'secondary' expansion of the pigment cells of the seeing larvae in darkness. Does the explanation of Babak hold here? And can it be admitted that impulses which owe their origin to the stimulus of darkness are set up in the retinae and conducted along nervous pathways to the pigment cells and there produce expansion? It may seem rather difficult to regard the absence of a stimulus (which continued darkness is) as a stimulus, or as the means of setting up a nervous impulse. But the 'secondary' expansion of the melanophores in darkness cannot be regarded simply as a relaxation on the part of the pigment cell, for then the blinded larvae would show it too. It seems necessary, therefore, to assume that nervous impulses started by chemical changes in the retinae due to the long continued absence of light are sent to the pigment cells along the pigment motor nerves causing them to expand.

The importance of the retinae in bringing about the secondary reactions is shown very well by the results of the experiments with the blindfolded larvae of *A. opacum*. When these larvae are exposed to bright diffuse light the same thing happens as in eyeless larvae—the melanophores become and remain permanently expanded as long as the larvae are kept in the light. The retinae being protected by the blindfolds no, or very little, light can reach them and cause changes leading to the formation of nerve impulses. But in darkness, since the retinae are present, although the eyes are blindfolded, the same thing happens as in normal larvae, where the long continued non-illumination of the retinae seems to be what causes the melanophores to expand secondarily.

According to Babak both the expanded and contracted states of the pigment cells are to be regarded as active, an impulse from the illuminated retina causing contraction, and one from the darkened retina causing expansion. Brücke was of the opinion,

in connection with his belief that light did not act directly on the pigment cells, but on special nerve endings which were reflexly connected with them, that the contracted condition was the one which resulted from stimulation. He was led to this opinion by the fact that when the nerves were cut the pigment cells expanded due to the falling away of the permanent tonic condition sustained by the nervous connections. When these cut nerves were stimulated the melanophores contracted. Keller ('95) easily corroborated these experiments and admitted that since electric stimulation brings about contraction, the contracted condition of the pigment cell must be regarded as the active state, which is brought about by stimulation. But non-illumination brings about in the pigment cells of the chameleon, this same result of contraction, and therefore the question is put by Keller as to whether a negative factor, such as the absence of light, can function as a stimulus. Keller is of the opinion that this is not, on a priori grounds, simply to be denied. He thinks, however, that light may be assumed to be the active stimulus and its effect consist in *inhibiting* the contracting impulse. Carlton claims that in *Anolis* the contracted condition of the melanophore represents the state of rest of the pigment cell, since it is produced by any means which brings the melanophores into an unstimulated state, viz., darkness, cessation of circulation, narcosis, nicotine poisoning, and possibly the cutting of nerves.

Brücke suggested three possibilities to explain the expanded and contracted states of the melanophores of the chameleon in relation to the question as to which could be regarded as the active state of the cell. Of these Keller (p. 160) finally leans toward the third. To quote from Keller:

Eine dritte Annahme würde darin bestehen, dass man beide Zustände als aktiv betrachtet, und von zwei antagonistisch wirkenden kontraktile Elementen ableitet, von denen das, welches den hellen Zustand hervorbringt, an Kräfte überwiegt, aber von den Empfindungsnerven aus nicht, wie das, welches den dunkeln Zustand bedingt, reflektorisch erregt werden kann.

But against the assumption of a double innervation which Bert, to satisfy the physiological conditions, suggests on purely

theoretical grounds, Keller (p. 161) points out that while both conditions of the pigment cell can be regarded as active this can be explained most simply by assuming a single innervation.

Für die akropetal wirkenden Elemente wäre eine in dem nie fehlenden Erregungszustande der Zelle begründete Art von Tonus anzunehmen, vermöge dessen sie in Kraft treten, wo ihre Antagonisten durch Trennung vom Nerven oder durch Chloroform ausser Tätigkeit gesetzt werden. Das Licht würde nicht auf dem Wege des Reflexes hemmend, sondern direkt erregend wirken.

This brings us to a consideration of the matter of the relative importance of the direct action of light and other stimuli on the melanophores compared with that of indirect stimulation. By direct stimulation is meant the action of the stimuli on the pigment cells themselves. From the results of the experiments carried out to determine the influence of the nervous system on the reactions of the melanophores it was seen that the pigment cells are under both sympathetic and spinal control, but also that when the central nervous system was completely destroyed the responses of the melanophores still took place. Biedermann holds that temperature acts directly on the pigment cells. Steinach ('91), working on *Rana temporaria* and esculents found when all connections, nerves, blood vessels and soft parts are cut away, that the skin of the leg still turns dark in darkness and pales when illuminated. Dutartre ('90) however saw that when the sympathetic and spinal nerves were cut, a permanent darkening of the skin. Hooker ('12) has given most conclusive evidence that light does act directly on the pigment cells. When he cut the lumbo-sacral plexus, or removed the sympathetic ganglia from which fibers are given off to the hind leg, the normal and synchronous changes in the pigment cells of *R. fusca* are not affected. When both of these operations are carried out, the part deprived of nerves still changes in color, but much more slowly for two days than the normally innervated leg; after this the normal reaction time is regained. By this is demonstrated, in this frog at any rate, that both spinal and sympathetic fibers control the movements of the melanophores. In further experiments, Hooker observed, when all the soft parts of

one leg are cut away, by which all the sympathetic fibers which may reach the pigment cells along the blood vessels are removed, and also in hanging drop cultures of small pieces of skin in plasma, that the pigment cells still respond to the stimulation of light. These responses are of particular interest. For the first day after the isolation of the pigment cells from nervous control, they react similarly to the pigment cells which are under this control, that is when illuminated they contract, and when placed in darkness they expand. But after the expiration of one day the responses are reversed, and upon illumination the cells expand, and on being placed in darkness they contract. If this secondary type of response came about immediately after the isolation of the pigment cells it would be in line with the assumption that has been made above to explain the behavior of the melanophores of *Amblystoma* when indirectly and directly stimulated, viz., that indirect stimulation by means of light caused contraction but that direct stimulation caused expansion. It would be very interesting to have Hooker's results corroborated on other animals, and it is to be hoped that an explanation of this reversal of the reactions of the isolated pigment cells will be soon forthcoming.

The results which have been described from isolated pieces of skin and for larvae in which the central nervous system has either been totally or partially destroyed show that direct stimulation of the pigment cells does take place. In eyeless larvae the possible action of light and darkness on the nerve endings in the skin cannot be denied, but the experiments which have been described show plainly, granting that the nerve endings are stimulated under normal conditions, that the changes in the melanophores come about when there is no possibility of the passage of impulses to the pigment cells. These experiments also give evidence for assuming that the primary responses shown by the normal larvae are brought about in greater measure by direct stimulation of the pigment cells themselves than by indirect stimulation through nerve endings in the skin; also that the indirect stimulation through the nervous system which is induced by impulses arising from the stimulation of the retinae, have

only a secondary influence. This influence, although secondary, must nevertheless be admitted to be a strong one, since it is, after a considerable time able to reverse the primary effect of the direct stimulation. That the effects of indirect stimulation are not altogether opposite to those of direct stimulation has already been shown by the fact that in the normal seeing larvae the primary responses to light and darkness come about more quickly than the corresponding changes in the eyeless larvae.

The mechanics of the movements of the pigment cells must be briefly referred to. The three chief views as to the real form and structure of pigment cells, and the means by which, and the manner in which, they regain their constant expanded pattern after contraction have only recently again been brought forward by as many investigators. Ballowitz ('13 and '14), to mention only two of his most recent papers on the subject, reiterates his many times expressed opinion that the movements of the pigment in the pigment cells are due to intracellular streaming in the chromatophores which have unchangeable cell forms. The protoplasm of the chromatophore is filled with numerous extremely fine, radially arranged, anastomosing canals in which the pigment is contained, and which are closed on the outside. No membrane can be demonstrated, but the walls of the canals are extremely thin and are formed by the protoplasm of the chromatophore. The contraction of the protoplasmic walls of the canals, alternating with its relaxation moves and drives forward the pigment granules. If the protoplasmic wall in the processes of the cells contracts in transverse waves from the periphery toward the center, then the pigment streams toward the center of the cell, and the canal protoplasm of the center of the cell disk at the same time relaxing, the pigment streams into it and rounds it off. On the other hand, if the central protoplasm contracts, and the protoplasm of the processes relaxes then the melanin granules stream out into the processes.

Spaeth ('13 a) holds to the theory, which is perhaps the most popular, that the chromatophores of fishes are fixed stellate cells within which the pigment carried in a rather fluid cytoplasm,

streams into and out of the processes during expansion and contraction.

Hooker ('14 b) upholds another and third view, that the melanophores of Amphibia (larval and adult *R. pipiens* and adult *R. fusca*) lie in preformed spaces and that they expand and contract within the spaces which enclose them. The acts of expansion and contraction, according to this theory, are brought about by pseudopodia, the pigment granules being carried in the cell cytoplasm and the pigment cells are therefore to be considered as amoeboid.

Not very much can be derived from a discussion as to which type of pigment cell (granting that in different classes of animals they all exist) fits best the requirements of direct and indirect stimulation. Though Ballowitz finds that the expansion and contraction of the chromatophores takes place when bits of skin containing them are isolated yet for the indirect stimulation of the type of cell described by him it would seem almost necessary to assume the existence of a double innervation of the cell, the simultaneous conduction of impulses along the two kinds of fibers causing at one time the contraction of one part of the cell and the expansion of the other. But on further consideration the assumption of a double innervation does not seem after all essential. Just as in a free bit of motile protoplasm, such as an *Amoeba* it is clear that a stimulus may cause one portion of the protoplasm to be contracted while the other is expanded (relaxed). In fact the movements of the pigment carrying portion of the amoeboid type of melanophore, whether it be a fixed stellate cell itself, or whether it be enclosed in a preformed space, conforms very nearly to what takes place in a free *Amoeba*, when directly stimulated and a single innervation of the pigment cell is all that is necessary to explain its movements under indirect stimulation.

Finally a few words may be said regarding the lengths of time taken by the pigment cells to change from one condition to another when stimulated by light. These have been already given under the general account of the reactions of the melanophores. Parker ('06) found that the distal migration of the pigment in

the chromatophores of *Phrynosoma blainvillei* is accomplished in fifteen minutes, while the proximal migration takes a little more than half an hour to be accomplished. Carlton ('03) found the same conditions to hold in *Anolis*, where the expansion of the melanophores was completed in four minutes, the contraction in twenty-five.

Babak ('10 a) has given the order in which the changes may be brought about in seeing and blinded Axolotls. He notes that in addition to the lack of the eyes, and the consequent loss of control over the movements of the pigment by the central nervous system, making the reactions of the melanophores exactly opposite to that observed in seeing larvae, the central nervous system also controls the amplitude (preventing extremes) and the rapidity (the indirect stimulation being faster than the direct) of the movements of the pigment. Furthermore, that illumination causes changes in the pigment cells to come about more quickly than darkness does, and the presence of the eyes more quickly than when they are removed. From these observations he set up the following order in which the changes in the pigment cells under the various conditions take place: (1) paling of normal seeing animals in the light (contraction); (2) darkening of blinded animals in the light (expansion) and the darkening of seeing animals in darkness (expansion); (3) paling of blinded animals (after long exposure to the light) in darkness (contraction).

The contracting of the melanophores when indirectly stimulated by light is then the quickest response, and when directly stimulated by darkness the slowest. In *A. punctatum* and *opacum* the reactions of the melanophores to light and darkness arrange themselves in quite a different sequence. In general it may be said that the seeing larvae respond more quickly to changes than do the eyeless, and that light, which primarily brings about expansion in both, acts more quickly than darkness, which primarily brings about contraction. The following list gives the sequence in which the melanophores under different conditions change (see table 2).

1. Expansion of the melanophores of seeing larvae in the light ($1\frac{1}{2}$ to 2 hours).

2. Expansion of the melanophores of eyeless larvae in light (2 to 3 hours), and contraction of the melanophores of seeing larvae in darkness (2 to 3 hours).

3. Contraction of the melanophores of eyeless larvae in darkness (4 to 5 hours).

4. 'Secondary' contraction of the melanophores of seeing larvae in the light (3 to 5 days), and 'secondary' expansion of the melanophores of seeing larvae in darkness (5 days or more).

SUMMARY

1. Normal seeing larvae and eyeless larvae of *Amblystoma punctatum* and *A. opacum* show different states of the melanophores under identical conditions of illumination and darkness, after they have been kept for some time under these conditions.

2. The melanophores of normal seeing larvae that have been kept for some time (longer than 3 to 5 days) in bright diffuse daylight on an indifferent background are contracted. The melanophores of eyeless larvae are maximally expanded. The melanophores of normal seeing larvae that have been kept for some time (longer than 5 days or more) in darkness are expanded. The melanophores of eyeless larvae are contracted.

3. The melanophores of normal seeing larvae that have been kept in bright diffuse daylight on a white background are contracted; on a black background, expanded.

4. The primary reactions of the melanophores of normal and eyeless larvae on the other hand, are identical, light bringing about an expansion, darkness a contraction. In normal larvae the response to light is complete in $1\frac{1}{2}$ to 2 hours, the response to darkness in two to three hours. In eyeless larvae these responses take two to three hours and four to five hours respectively. Only after being kept for some time under a particular condition do the secondary state of the melanophores (as given under paragraph 2) in the normal larvae take place, by which the melanophores assume the opposite state to that which they were in primarily.

5. The melanophores of blindfolded larvae, when illuminated, act like those of eyeless individuals—they expand and remain so. When placed in darkness they act like those of normal seeing larvae—at first they contract, but after five days or more they expand.

6. The responses of the melanophores of recently metamorphosed young adults are identical with those of the larvae, only slower and less complete.

7. The melanophores of very young larvae do not react to light, darkness and backgrounds, until certain stages of development have been reached (pp. 592–595).

8. The melanophores in isolated pieces of skin, aseptically removed and suspended in drops of various fluids, do not react to daylight, to the light from a Nernst glower, nor to darkness. But they do react to the light from an arc-lamp by contracting. From the work of others, (Hertel, Spaeth, and Torracca) it may be assumed that this contraction is caused, not by visible light but by the ultra-violet rays. A 0.01 per cent solution of chloretone inhibits this effect of the arc light.

9. Solutions of NaCl, KCl, and atropin have no expanding or contracting effect on the melanophores, neither when the larvae themselves are placed therein, nor when small pieces of skin are suspended in them. Atropin sulphate (1 per cent) and curare (0.2 per cent and 0.1 per cent) cause the melanophores to expand when larvae are placed in them, but do not affect the melanophores of isolated pieces of skin placed in 1 per cent to 4 per cent, and 0.1 per cent, 0.2 per cent and 1 per cent solutions respectively. Chloretone always produces an expansion of the melanophores, and inhibits the effects of light and darkness. Larvae cannot be kept in solutions of either NaCl or KCl without fatal results. In a Ringer solution opacum larvae will live for as long as twenty days, and the melanophores will respond in the normal ways to light and darkness.

10. Low temperatures cause the melanophores, of normal larvae and of larvae with destroyed central nervous systems, to expand, high temperatures (above 28°C.) causes them to contract. Below 12° to 15°C. the effect of darkness is inhibited, in

that the melanophores remain expanded. Above 15°C. the characteristic response of contraction takes place as at ordinary room temperatures, though more slowly, while the expanding effect of illumination is hastened. High temperatures (above 32°C.) hasten the contracting effect of darkness and retard the expanding effect of light. The melanophores of isolated pieces of skin when cooled below 10°C. incompletely expand and when warmed above 38°C. they incompletely contract. Chloretone inhibits the contracting effect of a high temperature.

11. The melanophores of *Amblystoma* larvae show no post-mortem changes and there is no 'center' for their contraction and expansion. The melanophores, are, however, under both spinal and sympathetic nerve control, the spinal control being relatively unimportant. The sympathetic fibers leave the spinal cord by means of the 1st or 2nd, possibly both, spinal nerves. When the spinal cord posterior to the 2nd nerve is destroyed, the primary and secondary reactions of the melanophores take place as in normal seeing larvae. When the portion of the central nervous system anterior to this level is destroyed, the secondary reactions no longer take place, though the primary reactions are perfectly normal.

12. The primary responses of the melanophores to light and darkness are due essentially to direct stimulation, though indirect stimulation through the eyes also plays a part. The secondary reactions of the melanophores of normal seeing larvae are due to nervous activities, set up by stimulation of the retinae, but in which supposed sensory endings in the skin have little or no share. The indirect stimulation through the retinae assists the direct, in that in larvae with eyes the primary reactions of the melanophores come about more quickly than in eyeless larvae. Only later is the result of the indirect stimulation opposite to that of the direct.

13. When larvae are placed in a 0.2 per cent solution of curare they are soon rendered immotile and the pigment cells remain expanded under all conditions. If a small amount of a 1 per cent solution is injected into the body cavity the larvae are again rendered immotile but the melanophores react to light and darkness as usual.

14. If a drop or two of a 1 per cent solution of strychnine is injected into the body cavity the melanophores soon contract after the cramp begins, which points to the existence of a spinal control.

15. If a few drops of a 0.01 per cent solution of nicotine is injected into the body cavity the primary reactions of the melanophores to light and darkness take place as usual.

16. An induced current of sufficient strength and duration causes the melanophores to contract in normal larvae, in larvae in which the central nervous system has been destroyed, in excised portions of the body and in isolated pieces of skin. A constant current causes the melanophores to expand.

17. Light, temperature, and electric currents all act directly on the melanophores. Light and electric currents in addition also act indirectly on the pigment cells by means of spinal and sympathetic nerves. The nerve endings for the reception of indirect light stimuli are in the retinae, sensory nerve endings in the skin probably playing no part.

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